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(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIALINFECTION

(57) Abstract: Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

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COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

TECHNIGAL FIELD

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. *Chlamydia trachomatis* is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. *Chlamydia trachomatis* may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with *Chlamydia trachomatis*, is the leading cause of preventable blindness worldwide. *Chlamydia pneumonia* is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to *Chlamydia pneumonia* have been shown to be at least twice as likely to suffer from coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical

compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments,, the polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a *Chlamydial* protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256, 292, and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known *Chlamydia* antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a *Chlamydial* protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more *Chlamydia* polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines for prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, Bcells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, within other aspects, methods for

removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of *Chlamydial* infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting *Chlamydia* infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting *Chlamydia* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

The present invention also provides methods for detecting *Chlamydia* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting *Chlamydia* infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe

comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone

1-B1-66.

SEQ ID NO: 2 is the determined DNA sequence for the C. trachomatis clone

4-D7-28.

SEQ ID NO: 3 is the determined DNA sequence for the C. trachomatis clone

3-G3-10.

SEQ ID NO: 4 is the determined DNA sequence for the C. trachomatis clone

10-C10-31.

SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66.

SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28.

SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 9 is a third predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 10 is a fourth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-B1-

66/48-67.

SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-B1-

66/58-77.

SEQ ID NO: 15 is the determined DNA sequence for the *C. trachomatis* serovar LGV II clone 2C7-8

SEQ ID NO: 16 is the determined DNA sequence for a first putative open reading frame from *C. trachomatis* serovar D

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the first putative open reading frame from *C. trachomatis* serovar D

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide CtC7.8-12

SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from *C. trachomatis* serovar D

SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from C. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from *C. trachomatis* LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from *C. trachomatis* LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from *C. trachomatis* LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from C. trachomatis LGV II

SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from C. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 29 is the determined DNA sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 30 is the predicted amino acid sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 31 is the amino acid sequence for a 10mer consensus peptide from CtC7.8-12 and CtC7.8-13

SEQ ID NO: 32 is the predicted amino acid sequence for clone 2C7-8 from C. trachomatis LGV II

SEQ ID NO: 33 is the determined DNA sequence of a clone from C. trachomatis serovar D which shows homology to clone 2C7-8

SEQ ID NO: 34 is the predicted amino acid sequence encoded by the sequence of SEQ ID NO: 33

SEQ ID NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer) from C. pneumonia

SEQ ID NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from C.

pneumonia

SEQ ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer) from C.

pneumonia

SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of *C. trachomatis*

SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of *C. pneumonia*

SEQ ID NO: 44 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II clone</u> 19783.3,jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 19783.4.jen.seq(1>481)CTL2#11-5', representing the 5' end.

SEQ ID NO: 46 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II clone</u>19784CTL2_12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

SEQ ID NO: 48 is a first determined DNA sequence for the <u>C. trachomatis</u> LGV II clone 19786.3,jen.seq(1>600)CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 19786.4,jen.seq(1>600)CTL2#18-5', representing the 5' end.

SEQ ID NO: 50 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19788CTL2_21consensus.seq(1>406)CTL2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19790CTL2 23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II clone</u> 19791CTL2_24consensus.seq(1>145)CTL2#24.

SEQ ID NO: 53 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis* LGV II clone 15-G1-89, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 56 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

SEQ ID NO: 60 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 11-H3-68, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the *C. trachomatis* LGV III clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene CT376 and to the glycogen hydrolase gene CT042.

SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

SEQ ID NO: 64 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II clone 11-A3-93</u>, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the *C. trachomatis*LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 66 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#6.

SEQ ID NO: 69 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#2.

SEQ ID NO: 71 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the <u>C. trachomatis</u>
<u>LGV II</u> clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 23509.1CtL2#3-3', representing the 3' end.

SEQ ID NO: 74 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 22121.1CtL2#10-3', representing the 3' end.

SEQ ID NO: 76 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the <u>C. pneumoniae LGV</u> <u>II</u> clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the <u>C. pneumoniae LGV</u>
<u>II</u> clone Cp_SWIB-His.

SEQ ID NO: 79 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

II clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

II clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

SEQ ID NO: 84 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

II clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 17-E11-72, sharing partial homology to the OppC_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

SEQ ID NO: 87 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

II clone 15-A3-26, sharing homology to the CT858 ORF.

SEQ ID NO: 89 is the determined amino acid sequence for the <u>C. pnuemoniae</u> clone Cp_SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the <u>C. trachomatis</u> LGV II clone CtL2_LPDA_FL.

SEQ ID NO: 91 is the determined amino acid sequence for the <u>C. pnuemoniae</u> clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the <u>C. trachomatis</u>
<u>LGV II</u> clone CtL2_TSA_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from C. trachomatis LGV II.

SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from C. trachomatis LGV II.

SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from C. trachomatis LGV II.

SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.

SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide from C. pneumonia.

SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from C. pneumonia.

SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from C. pneumonia.

SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide from C. pneumonia.

SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.

SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.

SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from *C. trachomatis*.

SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from C. trachomatis.

SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide from *C. pneumoniae*.

SEQ ID NO: 110 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.

SEQ ID NO: 111 is the determined DNA sequence for the <u>C. trachomatis</u>
<u>LGV II</u> clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

SEQ ID NO: 112 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-E2-9, sharing partial homology to the CT611 and CT 610 ORFs.

SEQ ID NO: 114 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the <u>C. trachomatis</u>

<u>LGV II</u> clone 20-G3-45, containing part of the pmpB gene CT413.

SEQ ID NO: 117 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.

SEQ ID NO: 118 is the determined DNA sequence for the <u>C. trachomatis</u>

<u>LGV II</u> clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar LGV II Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar LGV II</u> Cap1 gene CT529.

SEQ ID NO: 122 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar <u>E</u> Cap1 gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar E</u> Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar 1A</u> Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the \underline{C} . trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar G Cap1 gene CT529.

SEQ ID NO: 127 is the predicted full-length amino acid sequence for the <u>C.</u> trachomatis serovar <u>G</u> Cap1 gene CT529.

SEQ ID NO: 128 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the <u>C.</u> trachomatis serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 130 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar L1 Cap1 gene CT529.

SEQ ID NO: 131 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L1</u> Cap1 gene CT529.

SEQ ID NO: 132 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar L3</u> Cap1 gene CT529.

SEQ ID NO: 133 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L3 Cap1</u> gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar Ba</u> Capl gene CT529.

SEQ ID NO: 135 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar Ba</u> Capl gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the <u>C.</u> trachomatis serovar MOPN Cap1 gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar MOPN</u> Cap1 gene CT529.

SEQ ID NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of *C. trachomatis* serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #132-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 140 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-155 of *C. trachomatis* serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #146-163 of *C. trachomatis* serovar L2.

SEQ ID NO: 142 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #154-171 of *C. trachomatis* serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #162-178 of *C. trachomatis* serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 145 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #139-147 of *C. trachomatis* serovar L2.

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SEQ ID NO: 146 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #140-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-146 of *C. trachomatis* serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-145 of *C. trachomatis* serovar L2.

SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF peptide # F140->I of C. trachomatis serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Ga of C. trachomatis serovar L2.

SEQ ID NO: 151 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Gb of C. trachomatis serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 153 is the determined amino acid sequence for the peptide # 2 C7.8-7 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide # 2 C7.8-9 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide # 2 C7.8-10 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 170 is the determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 173 is the determined full-length DNA sequence for the *C. trachomatis* pmpC gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 175 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpl gene.

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SEQ ID NO: 176 is the predicted full-length amino acid sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpE gene.

SEQ ID NO: 178 is the predicted full-length amino acid sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 180 is the predicted full-length amino acid sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the C. trachomatis pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the *C. trachomatis* pmpC gene.

SEQ ID NO: 188 is the determined DNA sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the *C. trachomatis* pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpE gene in the SKB vaccine vector.

SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpE gene in the SKB vaccine vector.

SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpD gene in the pET17b vector.

SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpI gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpI gene in the pET17b vector.

SEQ ID NO: 224 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the C. pneumoniae Swib peptide 22-41.

SEQ ID NO: 229 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 27-46.

SEQ ID NO: 230 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 42-61.

SEQ ID NO: 231 is the determined amino acid sequence for the C. pneumoniae Swib peptide 46-65.

SEQ ID NO: 232 is the determined amino acid sequence for the C. pneumoniae Swib peptide 51-70.

SEQ ID NO: 233 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 56-75.

SEQ ID NO: 234 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 61-80.

SEQ ID NO: 235 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 66-87.

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SEQ ID NO: 236 is the determined amino acid sequence for the C. trachomatis OMCB peptide 103-122.

SEQ ID NO: 237 is the determined amino acid sequence for the C. trachomatis OMCB peptide 108-127.

SEQ ID NO: 238 is the determined amino acid sequence for the C. trachomatis OMCB peptide 113-132.

SEQ ID NO: 239 is the determined amino acid sequence for the C. trachomatis OMCB peptide 118-137.

SEQ ID NO: 240 is the determined amino acid sequence for the C. trachomatis OMCB peptide 123-143.

SEQ ID NO: 241 is the determined amino acid sequence for the C. trachomatis OMCB peptide 128-147.

SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.

SEQ ID NO: 243 is the determined amino acid sequence for the C. trachomatis OMCB peptide 137-156.

SEQ ID NO: 244 is the determined amino acid sequence for the C. trachomatis OMCB peptide 142-161.

SEQ ID NO: 245 is the determined amino acid sequence for the C. trachomatis OMCB peptide 147-166.

SEQ ID NO: 246 is the determined amino acid sequence for the C. trachomatis OMCB peptide 152-171.

SEQ ID NO: 247 is the determined amino acid sequence for the C. trachomatis OMCB peptide 157-176.

SEQ ID NO: 248 is the determined amino acid sequence for the C. trachomatis OMCB peptide 162-181.

SEQ ID NO: 249 is the determined amino acid sequence for the C. trachomatis OMCB peptide 167-186.

SEQ ID NO: 250 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-190.

SEQ ID NO: 251 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 171-186.

SEQ ID NO: 252 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 175-186.

SEQ ID NO: 252 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 175-186.

SEQ ID NO: 253 is the determined amino acid sequence for the *C. pneumoniae* OMCB peptide 185-198.

SEQ ID NO: 254 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 96-115.

SEQ ID NO: 255 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 101-120.

SEQ ID NO: 256 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 106-125.

SEQ ID NO: 257 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 111-130.

SEQ ID NO: 258 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 116-135.

SEQ ID NO: 259 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 121-140.

SEQ ID NO: 260 is the determined amino acid sequence for the C. trachomatis TSA peptide 126-145.

SEQ ID NO: 261 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 131-150.

SEQ ID NO: 262 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 136-155.

SEQ ID NO: 263 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

SEQ ID NO: 264 is the predicted full-length amino sequence for the *C. trachomatis* CT529/Cap 1 gene serovar I.

SEQ ID NO: 265 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 266 is the predicted full-length amino sequence for the $\it C$. $\it trachomatis$ CT529/Cap 1 gene serovar K.

SEQ ID NO: 267 is the determined DNA sequence for the *C. trachomatis* clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.

SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the C. trachomatis CT016 gene in clone 2E10.

SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the C. trachomatis tRNA syntase gene in clone 2E10.

SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the C. trachomatis clpX gene in clone 2E10.

SEQ ID NO: 271 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-30 representing the 5'end.

SEQ ID NO: 272 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-30 representing the 3'end.

SEQ ID NO: 273 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-28.

SEQ ID NO: 274 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-27.

SEQ ID NO: 275 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-26.

SEQ ID NO: 276 is the determined DNA sequence for the C. trachomatis clone CtL2gam-24.

SEQ ID NO: 277 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-23.

SEQ ID NO: 278 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-21.

SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis

clone CtL2gam-18.

SEQ ID NO: 280 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-17.

SEQ ID NO: 281 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-15 representing the 5' end.

SEQ ID NO: 282 is a second determined DNA sequence for the *C. trachomatis* clone CtL2gam-15 representing the 3' end.

SEQ ID NO: 283 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-13.

SEQ ID NO: 284 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-10.

SEQ ID NO: 285 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-8.

SEQ ID NO: 286 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-6 representing the 5' end.

SEQ ID NO: 287 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 3' end.

SEQ ID NO: 288 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-5.

SEQ ID NO: 289 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-2.

SEQ ID NO: 290 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-1.

SEQ ID NO: 291 is the determined full-length DNA sequence for the *C. pneumoniae* homologue of the CT529 gene.

SEQ ID NO: 292 is the predicted full-length amino acid sequence for the *C. pneumoniae* homologue of the CT529 gene.

SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

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DESCRIPTION OF THE FIGURES

Fig. 1 illustrates induction of INF- γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

- Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.
- Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).
- Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with *C. trachomatis* SWIB protein.
- Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.
- Fig. 6 illustrates the 5' and 3' primer sequences designed from *C. pneumoniae* which were used to isolate the SWIB and S13 genes from *C. pneumoniae*.
- Figs. 7A and 7B show induction of IFN-γ from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to *C. trachomatis* and *C. pneumonia* upon activation by monocytederived dendritic cells expressing chlamydial proteins.
- Fig. 8 shows the identification of T cell epitopes in Chlamydial ribosomal S13 protein with T-cell line TCL 8 EB/DC.
- Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against *C. pnuemoniae*-infected dendritic cells to recombinant *C. pneumonia*-SWIBprotein, but not *C. trachomatis* SWIB protein.
- Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.
- Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

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In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Chlamydia* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (*i.e.*, generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most

preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, 125I-labeled Protein A.

Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and

polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished, relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA*, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences specifically recited herein. "Substantial homology," as used herein, refers to polynucleotide sequences that are capable of hybridizing under moderately

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stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode a polypeptide that is the same as a polypeptide of the present invention.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy* – the *Principles and Practice of Numerical Taxonomy*, Freeman Press, San

Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks *Proc. Natl. Acad.*, Sci. USA 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamydia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and

Chlamydia pneumoniae. The antigens may thus be employed in a vaccine for both C. trachomatis genital tract infections and for C. pneumonia infections. Further characterization of these Chlamydia antigens from Chlamydia trachomatis and Chlamydia pneumonia to determine the extent of cross-reactivity is provided in Example 6. Additionally, Example 4 describes cDNA fragments (SEQ ID NO: 15, 16 and 33) isolated from C. trachomatis which encode proteins (SEQ ID NO: 17-19 and 32) capable of stimulating a Chlamydia-specific murine CD8+ T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamydia genomic or cDNA expression library by screening with a Chlamydia-specific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein.. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a *Chlamydia*-infected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem.* 80:116-132, 1967.

Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate *Chlamydia* cDNA or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known

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techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoter-primer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A

new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

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In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamydial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (i.e., an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a *Chlamydial* protein. Antisense technology can be used to control gene expression through triple-helix formation, which

compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (see Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

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A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

Any polynucleotide may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

As noted above, immunogenic portions of *Chlamydia* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a *Chlamydia* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *Chlamydia* antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an

expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known Chlamydial protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native Certain preferred fusion partners are both immunological and recombinant protein. expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the

fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in E. coli (thus functioning as

an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305. Additionally, the fusion protein Ra12 may be linked to the inventive polynucleotides to facilitate protein expression.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant,

such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *Chlamydia* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective) virus. Techniques for incorporating polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

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In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune response-modifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-Chlamydia effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with

immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting cells may be transfected or transduced with a polynucleotide sequence. wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipidmediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," Immunological Reviews, 157:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al.* (Crit. Rev. Oncol. Hematol., 22(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as IsolexTM System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive

polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigenspecific T cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, *Immunological Reviews*, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (i.e., vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen.

Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, Crit. Rev. Therap. Drug Carrier Systems 15:143-198, 1998. and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., Proc. Natl. Acad. Sci. USA 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219, 1994; Kass-Eisler et al., Proc. Natl. Acad. Sci. USA 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993.

Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available

as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Ribi ImmunoChem Research Inc. (Hamilton, MT) (see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555. Another preferred adjuvant is a saponin, preferably QS21, which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations

comprises an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets *Chlamydia*-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-*Chlamydia* effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs

(Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a

Chlamydial protein (or portion or other variant thereof) such that the Chlamydial polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a nonconjugated immunological partner, separately or in the presence of the polypeptide.

Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

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While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

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In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a Chlamydial protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

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In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

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The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20TM (Sigma Chemical Co., St. Louis, MO) may be employed. The

immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.g., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods

are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamydia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be

performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-*Chlamydia* antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10³

L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and

the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ⁹⁰Y, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion

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of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Carriers specific for radionuclide agents include Nos. 4,429,008 and 4,873,088). radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, For example, U.S. Patent No. 4,673,562, to Davison et al. discloses radionuclide. representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect *Chlamydia*-specific sequences in biological samples. DNA probes or primers

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comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-y in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

A randomly sheared genomic library of Chlamydia trachomatis LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 µl of RPMI 10% FBS. 10 µl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free E. coli and Chlamydia-specific T cells were Positive E. coli pools were identified by determining IFN-y production and proliferation of the T cells in response to the pools.

Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEQ ID NO: 1-4, respectively. Clone 1-B1-66 is approximately in region 536690 of the C. trachomatis genome (NCBI C.

trachomatis database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the *C. trachomatis* genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published sequence for S13 ribosomal protein from *Chlamydia trachomatis* (Gu, L. et al. *J. Bacteriology*, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and 10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEQ ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of *Chlamydia trachomatis* LGV II described above. A *Chlamydia*-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of *Chlamydia trachomatis* LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the *Chlamydia*-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

In further studies involving clone 4C9-18 (SEQ ID NO: 21), the full-length amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from *C. trachomatis* (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

To further characterize the open reading frame containing the T cell stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the Ndel/EcoRI site of the pET17b vector (Novagen, Madison, WI), referred to as clone 4C9-

TCT-1 T-cell line by standard proliferation assays.

18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into *E. coli*. Selective induction of the transformed *E. coli* with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, *E. coli* expressing the 26 kDa protein were titered onto 1 x 10⁴ monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 10⁴ T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-γ in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a *Chlamydia*-specific T-cell response against the lipoamide dehydrogenase sequence. Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the

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Subsequent studies to identify additional *Chlamydia trachomatis* antigens using the above-described CD4+ T-cell expression cloning technique yielded additional clones. The TCT-1 and TCL-8 *Chlamydia*-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the *Chlamydia trachomatis* LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to *Chlamydia pnuemoniae*. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of *C. pnuemoniae*.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEQ ID NO: 61), identified using the TCT-3 cell line, has two partial open reading

frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, (SEQ ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line, contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone 22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEO ID NO: 81), identified using the TCT-3 cell line, contains a 2,085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp 2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as part of

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the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SycE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of *Chlamydia trachomatis* contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpI. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a *Chlamydial* infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

Based on the *Chlamydia trachomatis* LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpI. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, London, England). Specifically, PmpC was subcloned into the JAL vector using the 5' oligo GAT AGG CGC GCC GCA ATC ATG AAA TTT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine vector following PCR amplification of the gene using the following oligos: 5' oligo- TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C

(SEQ ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEQ ID NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindIII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEQ ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEQ ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Several of the genes encoding the recombinant proteins, as described below, lack the native signal sequence to facilitate expression of the protein. Full-length protein expression of pmpC was accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CGA

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GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy terminus portion of the gene, pmpCcarboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' NheI/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC CTT AGA ATG TCA TAC GAG CAC CGC AG (SEQ ID PmpD was also expressed as two overlapping proteins. The pmpD-amino NO: 210). terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a six-histidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CCG GTT AGC (SEQ ID NO: 211), and the 3' oligo-CAG AGG TAC CTC AGC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEQ ID NO: 192). For expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG TGG (SEQ ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191). The pmpE insert also lacks the native signal sequence. PCR amplification of the gene under conditions well known in the art was performed using the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and the 3' oligo- CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional amino acid sequence at the amino end, namely, MASMTGGQQNGRDSSLVPHHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' NheI/3' Spel cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant – CT603 (the CT603 ORF is a homolog of CPn0778 from *C. pnuemoniae*). The TSA open reading frame in clone

14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 951 bp insert that contains part of the ORF for DT431, encoding for clpP_1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was shown to Clone 17-C10-31 (SEQ ID NO: 114), identified by the be part of the dnaK gene CT396 TCT-3 cell line, contains a 976 bp insert. This clone contains part of the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 (SEQ ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase{gyrA_2}) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary strand. Clone 22-B3-53 (SEQ ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1 vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEQ ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional *Chlamydia trachomatis* antigens were identified by serological expression cloning. These studies used sera pooled from several *Chlamydia*-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a *Chlamydial* infection, that is a mucosal humoral immune response. The following immunoreactive clones were characterized and the inserts containing *Chlamydia* genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ

ID NO: 289), CTL2gam-5 (SEQ ID NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

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EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

The ability of recombinant Chlamydia trachomatis antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., J. Immunology 157:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from C. trachomatis patients as well as from normal donors whose Tcells are known to proliferate in response to Chlamydia antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and $50\,\mu\text{g/ml}$ gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-y levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-γ production in a Chlamydia-specific T cell line used to screen a genomic library of C. trachomatis LGV II.

Further studies have identified a *C. trachomatis*-specific T-cell epitope in the ribosomal S13 protein. Employing standard epitope mapping techniques well known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were identified with a *Chlamydia*-specific T-cell line from donor CL-8 (T-cell line TCL-8 EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100% identical with the corresponding *C. pneumoniae* sequence, explaining the cross-reactivity of the T-cell line to recombinant *C. trachomatis*- and *C. pneumoniae*-rS13. The response to the second peptide

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rS13 56-75 (SEQ ID NO: 108) is C. trachomatis-specific, indicating that the rS13 response in this healthy asymptomatic donor was elicited by exposure to C. trachomatis and not to C. pneumoniae, or any other microbial infection.

As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed as described above, except that only peptides from C. trachomatis were tested. The T-cells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of C. trachomatis.

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEQ ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope mapping immunoassays were performed, as described above, to further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of *C. trachomatis* serovar LGVII.

EXAMPLE 3 PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

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EXAMPLE 4

ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

A genomic library of *Chlamydia trachomatis* LGV II was constructed by limited digests using BamHl, BgIII, BstYi and MboI restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helper-free Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The *Chlamydia* library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2^d restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-γ production using Elispot analysis (SEE Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the *Chlamydia*-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in a an additional positive clone, which

contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7.8 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of *Chlamydia trachomatis*, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as CT529 and/or the Cap1 gene) from serovar L2 was obtained by PCR amplification using 5'-tttttgaagcaggtaggtgaatatg (forward) (SEQ ID NO: 159) and 5'-ttaagaaatttaaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified *C. trachomatis* L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the *Eco*RI site of pBIB-KMS, a derivative of pBIB-KS for expression. The *Chlamydia pnuemoniae* homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 10⁵ IFU, essentially as described (Denamur, E., C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEQ ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEQ ID

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NO: 124, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 125); L1 (SEQ ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'-ggtataatatctctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaagcactttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-cettacacagtectgetgae (SEQ ID NO: 165) and a reverse primer 3'-gttteegggeeeteacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NI1), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2^d restricted target cells. In this assay, aliquots of P815 cells (H2^d) were labeled at 37° C for one hour with 100 μCi of ⁵¹Cr in the presence or absence of 1 μg/ml of the indicated peptides. Following this incubation, labeled P815 cells were washed to remove excess ⁵¹Cr and peptide, and subsequently plated

in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were added at the indicated effector:target ratios. Following a 4 hour incubation, supernatants were harvested and measured by gamma-counter for release of ⁵¹Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEO ID NO: 138-156 were synthsized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame.As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-147, SEQ ID NO: 139) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEQ ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2^d (K^d and L^d) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEO ID NO: 15) defines a gene which encompasses an antigen from Chlamydia capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against Chlamydia.

To confirm these results and to further map the epitope, truncated peptides (SEO ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEO ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the Chlamydia-specific T-cells.

Sequence alignments of Cap1 (CT529) from selected serovars of C. trachomatis (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the *Chlamydia* specific T-cells was compared. Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a ⁵¹Cr release assay, as described above. The *Chlamydia*-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1₁₃₉₋₁₄₇ is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2^d) cells were infected with *C. trachomatis* serovar L2 and tested to determine whether these cells are recognized by a CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with *C. trachomatis*. To determine if infection with *C. trachomatis* primes a Cap1#139-147 epitope-specific T-cell response, mice were infected i.p. with 10⁸ IFU of *C. trachomatis* serovar L2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard ⁵¹Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar L2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-

coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

EXAMPLE 5

GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH CHLAMYDIA ANTIGENS

Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ T-cell responses. DNA immunizations were intradermal at the base of the tail and polypeptide immunizations were administered by subcutaneous route. Results from standard ³H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEO ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with 10 µg purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5) formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by

standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, *Cancer Research*, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an *Chlamydia*-specific immune response.

In a further experiment, C3H mice were immunized at three separate time points at the base of the tail with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham, London, England). Antigen-specific antibody titers were measured by ELISA, showing both polypeptides induced a strong IgG response, ranging in titers from 1 x10⁻⁴ to 1 x10⁻⁵. The IgG1 and IgG2a components of this response were present in fairly equal amounts. Antigen-specific T-cell proliferative responses, determined by standard ³H-incorporation assays on spleen cells isolated from immunized mice, were quite strong for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000 cpm above the negative control). The IFNγ production was assayed by standard ELISA techniques from supernatant from the proliferating culture. *In vitro* restimulation of the culture with S13 protein induced high levels of IFNγ production, approximately 25 ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein also induced IFNγ, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 μg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigenspecific antibody responses were determined by standard ELISA techniques. Antigenspecific IgG antibodies were present in the blood of SWIB-immunized mice, with titers

ranging from 1 x10⁻³ to 1 x10⁻⁴, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes, as measured by IFNγ production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 serovar LGVII CTL epitope, defined by the CT529 10mer consensus peptide (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 µg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigen-specific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

EXAMPLE 6

EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes *Chlamydia trachomatis* as well as *Chlamydia pneumonia* infected monocyte-derived dendritic cells, suggesting *Chlamydia trachomatis* and *pneumonia* may encode cross-reactive T-cell epitopes. To isolate the *Chlamydia pneumonia* genes homologous to *Chlamydia trachomatis* LGV II

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clones 1B1-66, also referred to as SWIB (SEQ ID NO: 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with C. pneumonia strain TWAR (CDC/CWL-029). After three days incubation, the C. pneumoniainfected HeLa cells were harvested, washed and resuspended in 200 μ l water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the 3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The C. pneumonia-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from C. pneumonia were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively), a 15 kDa protein referred to as CpS13 (SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

EXAMPLE 7

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-γ PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

The ability of recombinant Chlamydia pneumoniae antigens to induce T cell proliferation and interferon-y production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., J. Immunology 157:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from C. pneumoniae patients as well as from normal donors whose T- 86

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cells are known to proliferate in response to Chlamydia antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 µg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 u g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 µl, 50 µl of medium is removed from each well for determination of IFN-y levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-y (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-y serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID

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NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic_cells cultures were washed and 2.5 x 10⁴ T cells (TCL-8) added and allowed to incubate for an additional 72 hours. The amount of INF-y in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-y, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To validate these results, the T cell epitope of C. trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the TCL-8 line. The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEO ID NO: 43) and C. trachomatis (SEQ ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding peptides described above (SEQ ID NO; 41-43).

Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis* did not respond to either *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during *in vivo C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ

ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a C. pneumoniae-SWIBspecific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10⁴ T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

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EXAMPLE 8

IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES AGAINST CHLAMYDIA ANTIGENS

The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. trachomatis. To characterize the immune responses of normal donors against chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. One donor, AD104, responded to recombinant C. pneumoniae elementary bodies. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific response. Three out of 12 donors had a C. trachomatis-SWIB, but not a C. pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae- S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a T-cell response in PBMC of normal study subjects.

Table I.

Immune response of normal study subjects against Orlanydia

onor	Sex	<i>Chlamydia</i> IgGtiter	CI EB	CP EB	CT Swib	CP Swib	CT Sl3	CP Sl3	Cl' lpdA	CT TSA
D100	male	negative	++	+++	+	-	++	++	-	nt
D104	female	negative	+++	++	-	-	-	++	-	nt.
D108	male	CP 1:256	++	++	+	+/-	+	+	+	nt
D112	female	negative	++	++	+		+	-	+/-	nt
D120	male	negative	-	+	-	-	-	-	-	nt
D124	female	CP 1:128	++	++	- ·	-	-	-	-	nt
D128	male	CP 1:512	+	++	-	-	++	+	++	-
D132	female	negative	#	++	-	-	+	+	-	-
D136	female	CP 1:128	+	++	-	-	+/-	-	-	-
D140	male	CP 1:256	++	++	-	-	+	+	-	-
D142	female	CP 1:512	++	# .	-	-	+	+	+	-
D146	female	negative	++	++	-	-	++	+	+	

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein. Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18h.

SI: Stimulation index

+/-:	SI ~	4
+:	SI>	4
++:	SI	10-30
+++:	SI >	30

In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to *C. trachomatis* by stimulating T-cells with *C. trachomatis* LGV II elementary bodies as previously described. Although the study subject was exposed to *C. trachomatis*, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against *C. trachomatis*. As shown in Fig. 10, a primary *Chlamydia*-specific T-cell line derived from donor CT-10 responded to *C. trachomatis*-SWIB, but not *C. pneumoniae*-SWIB recombinant proteins, confirming the exposure of CT-10 to *C. trachomatis*. Epitope mapping of the T-cell response to *C. trachomatis*-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEQ ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C. trachomatis patients. A summary of the patients' clinical profile and proliferative responses to various C. trachomatis and C. pneumoniae elementary bodies and recombinant proteins are summarized in Table II.

Proliferative response of C. trachomatis patients										
atients	Clinical manifestation	IgG titer		CP EB	CT Swib	CP Swib	CT S13	CP S13	CT lpdA	CT TSA
CT-1	NGU	negative	+	+	-	-	++	++	++	+
CT-2	NGU	negative	++	++	-	-	+	+/-	-	-
CT-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	-	-	-
CT-5	BV	Ct 1:256 Cp 1:256	++	++	-	-	+	-	-	-
CT-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	-	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	-	-	+	+	+	-
CT-8	Not known	Not tested	++	++	-	-	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-	-	++	+	+	-
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormał pap	Ct 1: 512	+++	+++	-	-	+++	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	-

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein
Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-

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incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18 hours.

SI: Stimulation index

+/-: SI ~ 4 +: SI > 4 ++: SI 10-30 +++: SI 30

Using the panel of asymptomatic (as defined above) study subjects and C. trachomatis patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from C. pneumoniae patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides, a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and S13, as well as . C. trachomatis lpdA and TSA are added in duplicate at concentrations of 0.5 to 10 μg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

Proliferative responses to the recombinant *Chlamydiae* antigens demonstrated that the majority of asymptomatic donors and *C. trachomatis* patients recognized the *C. trachomatis* S13 antigen (8/12) and a majority of the *C. trachomatis* patients recognized the *C. pneumonia* S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the *C. pneumonia* S13 antigen. Also, six out of twelve of the *C. trachomatis* patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of *C. trachomatis*. These results demonstrate that the *C. trachomatis* and *C. pneumonia* S13 antigen, *C. trachomatis* Swib antigen and the *C. trachomatis* lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to *Chlamydia* and an immune response elicited against them. This implies these antigens may

play a role in conferring protective immunity in a human host. In addition, the C. trachomatis and C. pneumonia S13 antigen is recognized equally well among the C. trachomatis patients, therefore indicating there may be epitopes shared between C. trachomatis and C. pneumonia in the S13 protein. Table III summarizes the results of these studies.

Table III.

Antigen	Normal Donors	C.t. Patients		
C.tSwib	3/12	0/12		
C.pSwib	0/12	0/12		
C.tS13	8/12	8/12		
C.pS13	4/12	8/12		
lpdA	4/12	6/12		
TSA	0/12	2/12		

A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from asymptomatic donors and *C. trachomatis* patients. Cellular immune responses were measured by standard proliferation assays and IFN-γ, as described in Example 7. Specifically, the majority of the antigens were in the form of single *E. coli* clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single *E. coli* clones were titered on 1 x 10⁴ monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 10⁴ T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after four days with a standard ³H-thymidine pulse for the last 18 hours. Induction of IFN-γ was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the *C. trachomatis* antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form *C. trachomatis* patients. In addition, proliferative responses were elicited from both the *C. trachomatis* patients and asymptomatic donors for

the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83 clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

Table IV.

Clone	C. t. Antigen	TCL from	TCL from	SEQ ID NO::
	(putative*)	Asymp. Donors	C. t. Patients	
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	groEL	1/2	4/4	111
22B3-53 (protein)	groEL	1/2	4/4	111
15H2-76 (E. coli)	PmpD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	rS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	dnaK	0/2	2/4	59
21C7-8 (E. coli)	dnaK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

EXAMPLE 9

PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal inoculation that uses a human isolate containing a strain of Chlamydia psittaci (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as Chlamydia trachomatis, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by Chlamydia trachomatis in women. In the first experiment, C3H mice (4 mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct/ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary/oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated mice showed no signs of tubal occlusion while negative control vaccinated groups had inflammatory cells in the lumen of the oviduct

In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing *C. trachomatis* SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made

intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of *C. psittaci* in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative control-immunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 1.75 for the vaccinated group versus 3. 75 for the control.

In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of C. psittaci or by injection of C. trachomatis serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. The degree of inflammation was scored as described above. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary/oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary /oviduct mean inflammation score of 8.

The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection with *C. psittaci*.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

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Claims

- 1. An isolated polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 5, 26, 32, 65, 90, 92-98, 103-108, 121, 123, 125, 127, 129, 131, 133, 135, 137, 175-180, 189-196, 264 and 266.
- 3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
- 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
 - A host cell transformed with an expression vector according to claim 4.
- 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.
- 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
- 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell

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transfected with a polynucleotide encoding the fusion protein.

- 9. A fusion protein according to claim 7, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.
- 10. A fusion protein according to claim 7, wherein the fusion protein comprises an affinity tag.
- 11. An isolated polynucleotide encoding a fusion protein according to claim 7.
- 12. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.
- 13. A pharmaceutical composition comprising a polypeptide according to claim 1, and a physiologically acceptable carrier.
- 14. A pharmaceutical composition comprising a polynucleotide molecule according to claim 3 and a physiologically acceptable carrier.
- 15. A pharmaceutical composition comprising a polypeptide and a physiologically acceptable carrier, wherein the polypeptide is encoded by polynucleotide molecule selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

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- and a physiologically acceptable carrier, wherein the polynucleotide molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 17. A pharmaceutical composition comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:
 - (a) a fusion protein according to claim 7;
 - (b) a polynucleotide according to claim 11; and
 - (c) an antibody according to claim 12.
- 18. A vaccine comprising a polypeptide according to claim 1, and an immunostimulant.
- 19. A vaccine comprising a polynucleotide molecule according to claim 3 and an immunostimulant.
- 20. A vaccine comprising a polypeptide and an immunostimulant, wherein the polypeptide is encoded by a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 21. A vaccine comprising a DNA molecule and an immunostimulant, wherein the DNA molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 122,

- 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 22. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:
 - (a) a fusion protein according to claim 7;
 - a polynucleotide according to claim 11; and (b)
 - an antibody according to claim 12. (c)
- 23. The vaccine of any one of claims 18-22 wherein the immunostimulant is an adjuvant.
- A method for inducing protective immunity in a patient, comprising 24. administering to a patient a pharmaceutical composition according to any one of claims 13-17.
- A method for inducing protective immunity in a patient, comprising 25. administering to a patient a vaccine according to any one of claims 18-22.
- An isolated polyclonal antibody, or antigen-binding fragment thereof, 26. that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.
 - A method for detecting Chlamydia infection in a patient, comprising: 27.
 - obtaining a biological sample from the patient; (a)
- contacting the sample with a polypeptide comprising an immunogenic (b) portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence

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recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291. (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and

- detecting the presence of antibodies that bind to the polypeptide. (c)
- A method for detecting Chlamydia infection in a patient, comprising: 28.
- obtaining a biological sample from the patient; (a)
- contacting the sample with a fusion protein comprising a polypeptide, (b) the polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - detecting the presence of antibodies that bind to the fusion protein. (c)
- The method of any one of claims 27 and 28 wherein the biological 29. sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.
- A method for detecting Chlamydia infection in a biological sample, 30. comprising:
- contacting the sample with at least two oligonucleotide primers in a (a) polymerase chain reaction, wherein at least one of the oligonucleotide primers is specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; and

- (b) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting *Chlamydia* infection.
- 31. The method of claim 30, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
- 32. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the sample with one or more oligonucleotide probes specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291; and
- (b) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe, thereby detecting *Chlamydia* infection.
- 33. The method of claim 32 wherein the probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
- 34. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i),

- and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
- 35. A method of detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
- 36. The method of any one of claims 34 and 35 wherein the binding agent is a monoclonal antibody.
- 37. The method of any one of claims 34 and 35 wherein the binding agent is a polyclonal antibody.
- 38. The method of any one of claims 34 and 35 wherein the biological sample is selected from the group consisting of whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.
 - 39. A diagnostic kit comprising:

- (a) a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (b) a detection reagent.
 - 40. A diagnostic kit comprising:
- (a) a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (b) a detection reagent.
- 41. The kit of claims 39 or 40 wherein the polypeptide is immobilized on a solid support.
- 42. The kit of claims 39 or 40 wherein the detection reagent comprises a reporter group conjugated to a binding agent.
- 43. The kit of claim 42 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.

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- The kit of claim 42 wherein the reporter group is selected from the 44. group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.
- A diagnostic kit comprising at least two oligonucleotide primers, at 45. least one of the oligonucleotide primers being specific for a polynucleotide molecule comprising a polynucleotide sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
- A diagnostic kit according to claim 43, wherein at least one of the 46. oligonucleotide primers comprises at least about 10 contiguous nucleotides of a sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
- A diagnostic kit comprising at least one oligonucleotide probe, the 47. oligonucleotide probe being specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
- A kit according to claim 47, wherein the oligonucleotide probe 48. comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291.
 - A diagnostic kit comprising: 49.
- at least one antibody, or antigen-binding fragment thereof, (a) according to claim 22; and
 - a detection reagent. (b)

- 50. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
 - (a) obtaining peripheral blood cells from the patient;
- (b) incubating the cells in the presence of at least one polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
 - (c) administering to the patient the proliferated T cells.
- 51. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
 - (a) obtaining peripheral blood cells from the patient;
- (b) incubating the cells in the presence of at least one polynucleotide, comprises a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 1-4, 15, 16, 21-25, 27, 29, 33, 44-64, 66-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
 - (c) administering to the patient the proliferated T cells.
- 52. The method of any one of claims 50 and 51 wherein the step of incubating the T cells is repeated one or more times.
- 53. The method of any one of claims 50 and 51 wherein step (a) further comprises separating T cells from the peripheral blood cells, and the cells incubated in step (b) are the T cells.

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- 54. The method of any one of claims 50 and 51 wherein step (a) further comprises separating CD4+ cells or CD8+ T cells from the peripheral blood cells, and the cells proliferated in step (b) are CD4+ or CD8+ T cells.
- 55. The method of any one of claims 50 and 51 wherein step (a) further comprises separating gamma/delta T lymphocytes from the peripheral blood cells, and the cells proliferated in step (b) are gamma/delta T lymphocytes.
- 56. The method of any one of claims 50 and 51 wherein step (b) further comprises cloning one or more T cells that proliferated in the presence of the polypeptide.
- 57. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 58. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 59. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) incubating antigen presenting cells in the presence of at least one
 polypeptide of claim 1;
 - (b) administering to the patient the incubated antigen presenting cells.
- 60. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) introducing at least one polynucleotide of claim 3 into antigen presenting cells;
 - (b) administering to the patient the antigen presenting cells.

- 61. The method of claims 59 or 60 wherein the antigen presenting cells are selected from the group consisting of dendritic cells. macrophage cells, B cells fibroblast cells, monocyte cells, and stem cells.
- 62. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 63. A pharmaceutical composition for the treatment if *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 64. A polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said immunogenic portion comprises a sequence of SEQ ID NO: 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256.
- 65. An immunogenic epitope of a *Chlamydia* antigen, comprising a sequence of SEQ ID NO: 31, 98, 106, 108, 138-140, 158, 167, 168, 246, 247 or 254-256.
- 66. An isolated polypeptide comprising a sequence recited in any one of SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256 and 292.

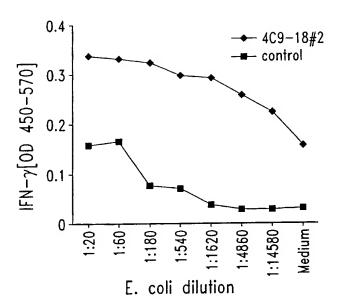


Fig. 1

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Fig. 2

Chlamydia C17.8 Peptide Screen

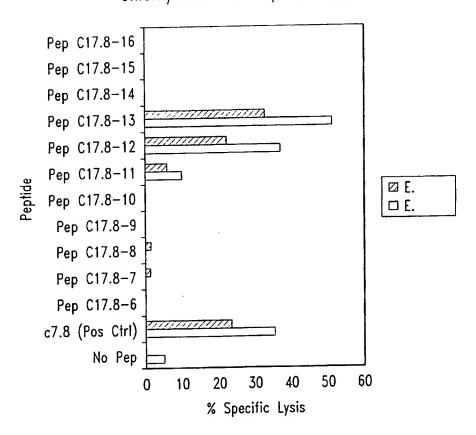
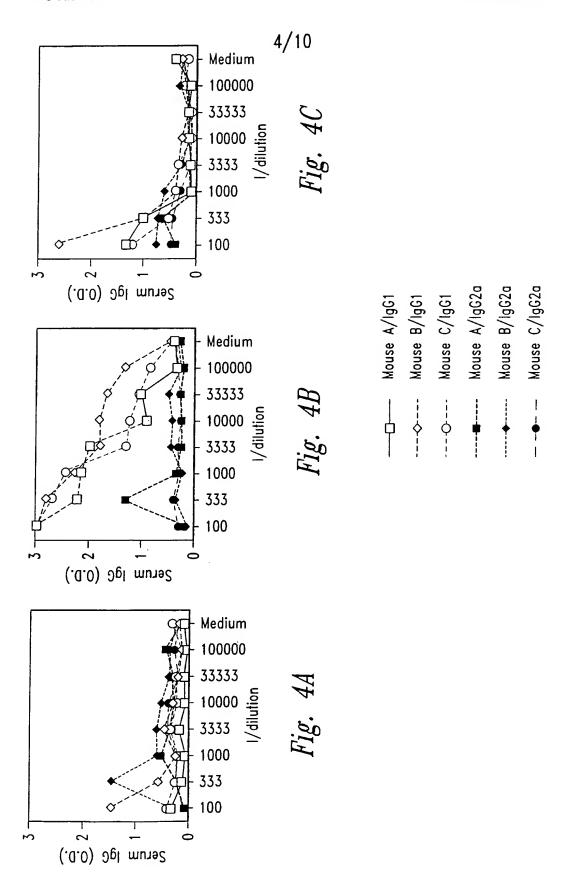
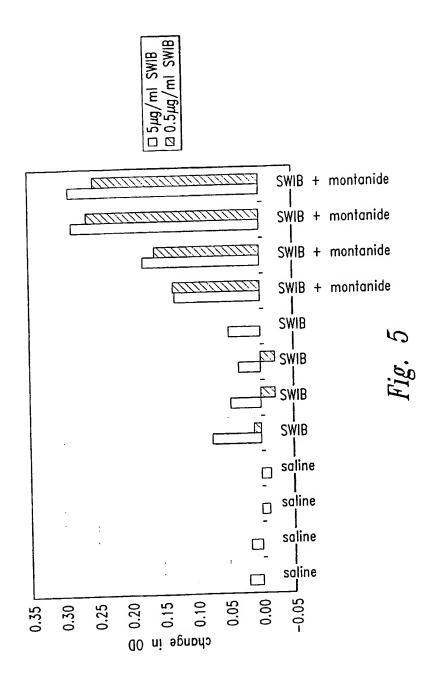


Fig. 3





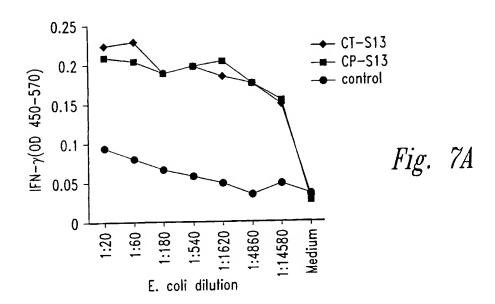
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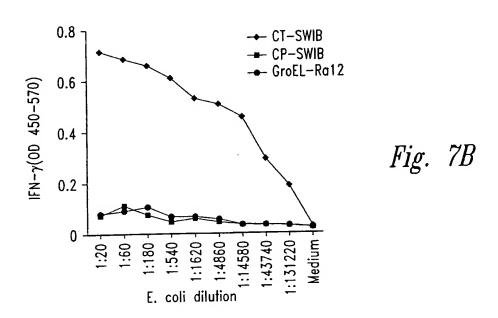
CP S13 Nde (5' primer)
5' GATATACATATGCATCACCATCACCATCACATGCCACGCATCATTGGAATGAT

CP S13 EcoRI (3' primer)
5' CTCGAGGAATTCTTATTTCTTCTTACCTGC

Fig. 6

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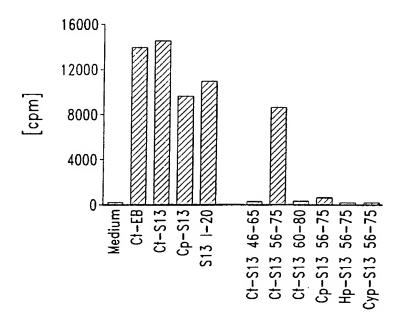
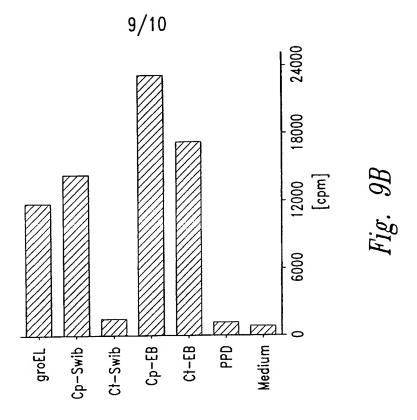
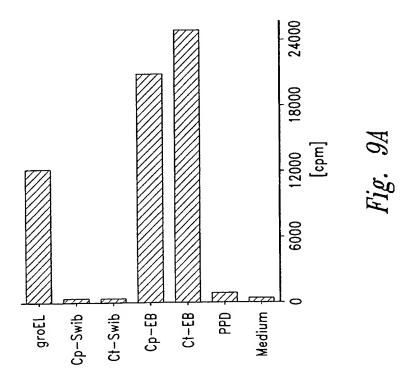
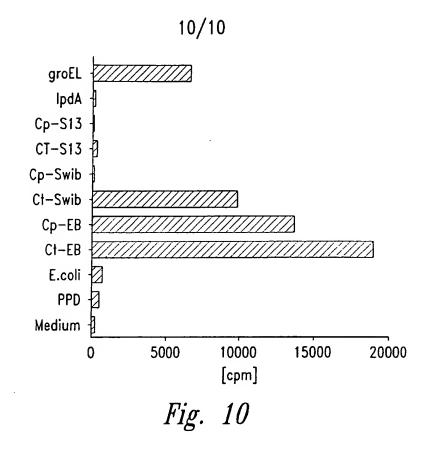


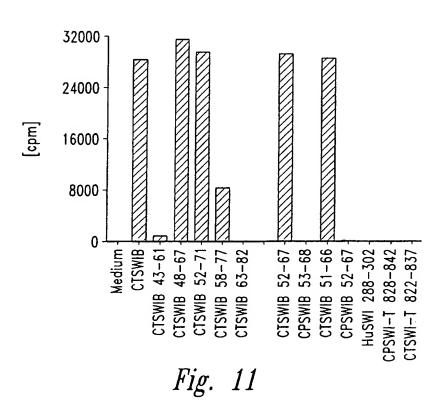
Fig. 8

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SUBSTITUTE SHEET (RULE 26)

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 Glu
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 Inc</td

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  atgaacgcgg agtcatccct accgatgcca caatgcgcac aaacgtacct aacatttatg
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120

180

240 300

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      <211> 600
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  cccaggaaca ttgaaacttt attaggagga actgaaatag gaaaattcac agtcacaccc 180
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PCT/US99/29012

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Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu

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Gly Thr Cys Leu Asn Arg Gly Cys Ile Pro Ser Lys Ala Leu Leu Ala

Gly Ala Glu Val Val Thr Gln Ile Arg His Ala Asp Gln Phe Gly Ile

His Val Glu Gly Phe Ser Ile Asn Tyr Pro Ala Met Val Gln Arg Lys 90

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Ser Asn Lys Ile Thr Val Phe Ser Gly Arg Gly Ser Leu Ile Ser Ser 120

Thr Glu Val Lys Ile Leu Gly Glu Asn Pro Ser Val Ile Lys Ala His 135 130

Ser Ile Ile Leu Ala Thr Gly Ser Glu Pro Arg Ala Phe Pro Gly Ile 155 150

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Ser Leu Leu Gln Ser Glu Tyr Thr Val Glu Gly Asp Leu Arg Arg 65 70

Val Gln Ser Asp Ile Lys Arg Leu Ile Ala Ile His Ser Tyr Arg Gly 85

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Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Leu Gly Cys Ser 70

Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp

Ala Gly Gly Ile Glu Gly Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser

Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 120

Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His 135

Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu

Arg Ile Leu Asp Ser Leu Ile Phe Phe Glu Asn His Gly Met Val Cys 165 170

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WO 00/34483 PCT/US99/29012

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185 Val Gly Ser Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala 200

195

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								Arç												
			Al	a I				Gly 295												
Leu	ı S	er	11	e I	eu	Ala	Gly	29: Glu	ı Gly	y S	er	Val	Let 31:	1 P.	he	Gln	Ası) AS	n	ser 320
305 Glr	i 1 A	rg	Th	r S	Ser	Asp	310 Gl:	o n Gly	y Le	u V	/al	Arg	Ası	n A	la	Ile	Ту	r Le	u :	Xaa
								c Se:												
								l Gl:												
			Se	r				a Se 37												
								31 n Th O												
							G1	u Gl												
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						. Le		r Al		0	Ser	Le								
			ıI					a Gl	ly Ti	nr										
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							s A	70 La Pi					r I	le						
							p G	lu A				c G1	u A							
						n As		le P			Let	ı Ti								
н	is	Le	u F	15 Iis	Le	u Pr	o A	sp G	ly A	20 sn	Let	u Se	er S	er	Hi:	s Ph	ne G	ly T	yr	Gl
							ne S	er T					er A	sp						
								50 ro L				r V	al P							
								sn T												

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585
Ala Val Gln Ser Met Ile Asn Thr Thr Ala His Gly Gly Ala Tyr Leu
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Phe Gly Thr Trp Gly Ser Ala Val Ser Asn Leu Phe Tyr Val His Asp
           615
                               620
Ser Ser Gly Lys Pro Ile Asp Asn Trp His His Arg Ser Leu Gly Tyr
                630
                      635
Leu Phe Gly Ile Ser Thr His Ser Leu Asp Asp His Ser Phe Cys Leu
                  650 655
             645
Ala Ala Gly Gln Leu Leu Gly Lys Ser Ser Asp Ser Phe Ile Thr Ser
         660 670
Thr Glu Thr Thr Ser Tyr Ile Ala Thr Val Gln Ala Gln Leu Ala Thr
                        680
Ser Leu Met Lys Ile Ser Ala Gln Ala Cys Tyr Asn Glu Ser Ile His
                 695
                                     700
Glu Leu Lys Thr Lys Tyr Arg Ser Phe Ser Lys Glu Cly Phe Gly Ser
Trp His Ser Val Ala Val Ser Gly Glu Val Cys Ala Ser Ile Pro Ile
                             730
             725
Val Ser Asn Gly Ser Gly Leu Phe Ser Ser Phe Ser Ile Phe Ser Lys
                          745
       740
Leu Gln Gly Phe Ser Gly Thr Gln Asp Gly Phe Glu Glu Ser Ser Gly
                       76C
                                        765
Glu Ile Arg Ser Phe Ser Ala Ser Ser Phe Arg Asn Ile Ser Leu Pro
                   775 780
Ile Gly Ile Thr Phe Glu Lys Lys Ser Gln Lys 'Thr Arg Thr Tyr Tyr
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                                 795
Tyr Phe Leu Gly Ala Tyr Ile Gln Asp Leu Lys Arg Asp Val Glu Ser
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                             810
Gly Pro Val Val Leu Leu Lys Asn Ala Val Ser Trp Asp Ala Pro Met
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Ala Asn Leu Asp Ser Arq Ala Tyr Met Phe Arg Leu Thr Asn Gln Arg
       835 840
Ala Leu His Arg Leu Gln Thr Leu Leu Asn Val Ser Cys Val Leu Arg
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865
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Pro Tyr Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala
                          25
Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro
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. 40

Leu S															
Gly H	is S				Phe (Glu				/ _					-
65 Ala L				Ser.	Ala				70						
Lys G			Ser	Phe				1117							
Ala A			Thr				120					120			
Pro S	Ser .	Asn				1 7 5					740				
Asn (3lu				1 - 0					122					
Ala :									. / 1/						
Val 1			400					יאו	3						
Val '							- 200)				200			
		Val				つてち					220				
Glγ	Gln				າາກ					23:	Glu G				
Ser				245						J	Gly				
			200	Ile				2n	-		l Ala				
			Leu	Phe			') H (1)				20	•		Ala
						201	_				300	,			Tyr.
															Ser 320
Asn				225					3.3	U					
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			_				4 h	• • • • • • • • • • • • • • • • • • • •				30.	_		e Ala
															Leu
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				4 0 1	_				4.1	LU					
			4.7	^				4.	45					•	a Lys
			_				44	111					_		n Gly
	_	n Gl	n Pr			40	55				40				p Gly
	ı Gl	у Ту			47	Λ				4	, ,				r Leu 480
Ту	r Gl	n As	sn Va	al Th	r Il	e Gl	lu G	ln G	ly A	rg I	le Va	ıl L∈	u Ar	g Gl	u Lys

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Ala	Lys	Leu	Ser 500		Asn	Ser	Leu	Ser 505	Gln	Thr	Gly	Gly	Ser 510	Leu	Tyr
Met	Glu	Ala 515	Gly	Ser	Thr	Leu	Asp 520	Phe	Val	Thr	Pro	Gln 525	Pro	Pro	Gln
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		675			Ser		680					685			
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	770			•	Leu	775			_		780				
785	_	-			790 His					795					800
				805					810					815	
			820		Ile			825					830		
		835			Ala		840					845			
	850				Gln	855					860				
865			_	_	870 Val					875					880
				885	Ser	_			890					895	
			900		Thr			905					910		
_		915		-			920					925			

Trp Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Val Arg 935 Gly Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His 955 950 Gly Arg Tyr Glu Tyr Arg Asp Ala Ser Arg Gly Tyr Gly Leu Ser Ala 970 965 Gly Ser Lys Val Xaa Phe 980 <210> 177 <211> 964 <212> PRT <213> Chlamydia Met Lys Lys Ala Phe Phe Phe Leu Ile Gly Asn Ser Leu Ser Gly <400> 177 1 5 Leu Ala Arg Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val 25 20 Pro Asp Pro Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly 40 Asp Thr His Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile 60 55 Leu Ala Ile Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile 70 75 Thr Asp Tyr Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe 85 90 Ala Lys Asn Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser 105 Pro Asn Ser Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile 120 125 Phe Glu Asn Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr 130 135 140 Ala Ala Asp Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu 155 Tyr Ile Asn His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser 170 175 165 Tyr Val Gln Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser 185 190 Glu Asn Gln Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr 200 205 Asn Thr Ala Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser 215 220 Phe Glu Ser Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys 230 235 Ala Gly Gly Ala Ile Phe Ser Pro Ile Cys Ser Leu Thr Gly Asn Arg 245 250 255 Gly Asn Ile Val Phe Tyr Asn Asn Arg Cys Phe Lys Asn Val Glu Thr 270 265 Ala Ser Ser Glu Ala Ser Asp Gly Gly Ala Ile Lys Val Thr Thr Arg 280 285 Leu Asp Val Thr Gly Asn Arg Gly Arg Ile Phe Phe Ser Asp Asn Ile 300 295 Thr Lys Asn Tyr Gly Gly Ala Ile Tyr Ala Pro Val Val Thr Leu Val 315 310

Asp	Asn	Gly	Pro	Thr 325	Tyr	Phe	Ile	Asn	Asn 330	Ile	Ala	Asn	Asn	Lys 335	
Gly	Ala	Ile	Tyr 340		Asp	Gly	Thr	Ser 345		Ser	Lys	Ile	Ser 350	Ala	
Arg	His	Ala 355		Ile	Phe	Asn	Glu 360		Ile	Val	Thr	Asn 365			Asn
Ala	Asn 370		Thr	Ser	Thr	Ser 375		Asn	Pro	Pro	Arg 380		Asn	Ala	Ile
Thr 385	Val	Ala	Ser	Ser	Ser 390	Gly	Glu	Ile	Leu	Leu 395	Gly	Ala	Gly	Ser	Ser 400
Gln	Asn	Leu	Ile	Phe 405	Tyr	Asp	Pro	Ile	Glu 410	Val	Ser	Asn	Ala	Gly 415	
Ser	Vai	Ser	Phe 420	Asn	Lys	Glu	Ala	Asp 425	Gln	Thr	Gly	Ser	Val 430	Val	Phe
Ser	Gly	Ala 435	Thr	Val	Asn	Ser	Ala 440	Asp	Phe	His	Gln	Arg 445	Asn	Leu	Gln
	450					455				Asn	460			_	
465	_				470					Phe 475					480
				485		_			490	Ser				495	_
	_	_	500					505		Thr			510		
		515					520			Ala		525			
	530					535				Tyr	54 0				
545					550					Ser 555					560
				565					570	Thr				575	
			580					585		Asp			590		
		595					600			Pro		605			
	610	_		_	-	615				Gln	620				
625					630	_			_	Ala 635					640
				645					650	Tyr Trp				655	
	_		660					665		Leu	_		670		
		675					680			Gly		685			
	690					695	_	_		Met	700				
705		_			710		_			715 Thr					720
		_		725		_			730	Tyr				735	
			740					745		Met			750		
		-1-		- 1 -		-,-		1							

760 Glu Gly Phe Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp 755 770 775 780 His Asn Cys His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln 785 Gly Thr Phe Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu 815 Pro Met Lys Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu 820 830 Gly Ala Leu Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly 835 840 945 Ala Tyr Pro Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu 850 855 Val Pro Ile Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro 870 Gln Ala Trp Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln 885 890 Glu Pro Gly Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe 900 905 Gly Ser Gly Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser 915 920 925 Gln Gln Thr Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His 930 935 940 Gly Phe Tyr Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile 950 955 Ala Leu Arg Phe <21C> 178 <211> 1530 <212> PRT <213> Chlamydia Met Ser Ser Glu Lys Asp Ile Lys Ser Thr Cys Ser Lys Phe Ser Leu 10 Ser Val Val Ala Ala Ile Leu Ala Ser Val Ser Gly Leu Ala Ser Cys 1 5 25 Val Asp Leu His Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val 40 Gly Pro Gln Ala Val Leu Leu Leu Asp Gln Ile Arg Asp Leu Phe Val 55 Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly 75 Asp Pro Ser Ser Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys 70 90 Val Glu Gln Ser Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln 105 100 Gly Val Asp Gln Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser 125 120 Phe Thr Ser Ser Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu 135 140 Gly Ile Ala Phe Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr 150 155 Asp Val Lys Ala Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp

				165					170					175	
Leu	Ile	Phe	Glu 180		Ile	Lys	Gly	Gly 185			Phe	Ala	Ser 190		
Ser	Leu	Glu 195		Gly	Gly	Ala	Cys 200		Ala	Gln	Ser	Ile 205	Leu	Ile	His
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Glu 225	Gly	Ser	Ser	Ala	Asn 230	Asp	His	Leu	Gly	Phe 235	Gly	Gly	Gly	Ala	Phe 240
Phe	Val	Thr	Gly	Ser 245	Leu	Ser	Gly	Glu	Lys 250	Ser	Leu	Tyr	Met	Pro 255	Ala
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	-			325					330				Gly Gly	335	
_		_	340					345					350 Glu		
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Ala	Ile	Ala	Ala	Gln 405	Glu	Ile	Val	Ser	11e 410	Gln	Asn	Asn	Gln	Ala 415	Gly
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Ser	Gly	Tyr	Ser	Gly 565	Gly	Gly	Ala	Ile	Leu 570	Gly	Arg	Glu	Val	Ala 575	Ile
			580					585			_		Gln 590		
Glu	Glu	Glu 595	Ala	Thr	Leu	Leu	Gly 600	Cys	Cys	Gly	Gly	Gly 605	Ala	Val	His

and the Cly
Gly Met Asp Ser Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly
610 Gly Val Ser Gly Gly Ala Leu Leu Ser
Asn Asn Tyr Ala Met Gly Gin Gly 635 640
625 630 633 Lys Thr Val Gln Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn 650 655
Lys Thr Val Gin Let Ald 327 650 655
645 11e Ala Ser Leu Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys 665 670
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Glu Leu Val Asp Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg
680 685 680 Figure 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
675 Val Tyr Gly Gly Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser 700
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Glu Gln Lys Asp Ash Ash Glu 200 745
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Ser Phe Tie III And 760 765
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Gly Gln Ile Pro Glu van hed 110 000 845
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Phe Ser Gly Ash Ser Ser Ser Ser Thr Cly
855 Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr Ile Ser Gln Asn Thr Gly 875 880
875 870 875 Car Cly Gly Ala Val Arg
865 870 875 Asn Val Ala Cys Ser Gly Gly Ala Val Arg Asn Val Leu Phe Tyr Asn Asn Val Ala Cys Ser Gly Gly Ala Val Arg
890 885 11e Clu Ala Phe Gly Gly Asp Ile
885 Ile Glu Asp His Gly Asn Val Leu Leu Glu Ala Phe Gly Gly Asp Ile 905 910
900 Val Phe Lys Gly Asn Ser Ser Phe Arg Ala Gln Gly Ser Asp Ala Ile 920 925
Val Phe Lys Gly Ash Ser Ser File 125
915 920 915 Tyr Phe Ala Gly Lys Glu Ser His Ile Thr Ala Leu Asn Ala Thr Glu 940
Tyr Phe Ala Gly Lys Gld 201 940
Oly Hig Ala The Val Phe His Asp Ala Leu Val Phe Glu Ash Leu Lys
GIV HIS AIR THE TOTAL STATE OF THE STATE OF
945 950 950 955 Ser Arg Glu Asn Pro Glu Arg Lys Ser Ala Glu Val Leu Leu Ile Asn Ser Arg Glu Asn Pro 970 975
965 970 Ser Lys Val Pro
965 Gly Tyr Thr Gly Ser Ile Arg Phe Leu Glu Ala Glu Ser Lys Val Pro 985 990
985 980 985 Leu Glu Leu Leu Asn Gly Ala
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995 1000 Thr Leu Cys Ser Tyr Gly Phe Lys Gln Asp Ala Gly Ala Lys Leu Val 1020
Thr Leu Cys Ser Tyr Gly Phe Lys Gin 1020
1010 Lys The Leu Asp Ser Gly Thr Plo Val
Leu Ala Ala Gly Ser Lys Leu Lys 110 25 1035 1040
1025 1030 1033 Gln Gly His Ala Ile Ser Lys Pro Glu Ala Glu Ile Glu Ser Ser Ser
GILL GIA UID WIN TOO -

				1045	;				1050)				1055	5
Glu	Pro	Glu	Gly 1060	Ala		Ser	Leu	Trp	Ile		Lys	Asn	Ala 1070		Thr
Thr	Val	Pro 1075	Met	Val	Asp		His 1080	Thr		Ser	Val	Asp 1085		Ala	Ser
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Ser	Ala	Glu 1159		Ser	Asn	L€u	Ser 1160		Ser	Asp	Leu	Gln 1169		His	Val
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Ser 1185	Glu	Ala	Lуз	Ile	Gln 1190		Gly	Thr	Leu	Val 1199		Asn	Trp	Asn	Pro 1200
Thr	Gly	Tyr	Arg	Leu 120!		Pro	Gln	Lys	Ala 1210		Ala	Leu	Val	Phe 121	
Ala	Leu	Trp	Glu 122	Glu		Ala	Val	Leu 122		Ala	Leu	I.ys	Asn 123		Arg
Phe	Ala	His 123	Asn	Leu	Thr	Ala	Gln 124		Met	Glu	Phe	Asp 1249		Ser	Thr
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Ala	Gly	Val	Asp	Ile 128		Leu	Met	Glu	Asp 129		Val	Leu	Gly	Val 1299	
_			130					130	5				1310)	
Val	Ser	Arg		Gly	Val	Val	Gly 132		Val	Tyr	Thr	Gly 132		Leu	Ala
	133	0		Phe		133	5				1.34	3			
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Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu 1495 Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr **151**5 1505 1510 Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 1525 <210> 179 <211> 1776 <212> PRT <213> Chlamydia <400> 179 Ala Ile Met Lys Phe Met Ser Ala Thr Ala Val Phe Ala Ala Val Leu 1 5 10 15 Ser Ser Val Thr Glu Ala Ser Ser Ile Gln Asp Gln Ile Lys Asn Thr 25 Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln Ala Phe Thr 40 Asp Met Met Leu Ala Asp Asn Thr Glu Tyr Arg Ala Ala Asp Ser Val 50 55 Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arg Lys His Leu 65 70 75 Ser Ser Ser Ser Glu Ala Ser Pro Thr Thr Glu Gly Val Ser Ser Ser 85 90 Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala Pro Ser Ser 100 105 110 Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn Gly Gly Ile 115 120 125 Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln Asp Ser Leu 135 140 Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe Gly Glu 150 155 160 Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn Gly Gly Ala 165 170 175 Ile Tyr Gly Glu Lys Glu Val Val Phe Glu Asn Ile Lys Ser Leu Leu 185 Val Glu Val Asn Ile Ser Val Glu Lys Gly Gly Ser Val Tyr Ala Lys 200 205 195 Glu Arg Val Ser Leu Glu Asn Val Thr Glu Ala Thr Phe Ser Ser Asn 215 220 Gly Gly Glu Gln Gly Gly Gly Ile Tyr Ser Glu Gln Asp Met Leu 230 Ile Ser Asp Cys Asn Asn Val His Phe Gln Gly Asn Ala Ala Gly Ala 250 255 245 Thr Ala Val Lys Gln Cys Leu Asp Glu Glu Met Ile Val Leu Leu Thr 265 270 Glu Cys Val Asp Ser Leu Ser Glu Asp Thr Leu Asp Ser Thr Pro Glu 285 275 280 Thr Glu Gln Thr Lys Ser Asn Gly Asn Gln Asp Gly Ser Ser Glu Thr 290 295 300 Lys Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser Pro Asp 305 310 Asp Val Leu Gly Lys Gly Gly Gly Ile Tyr Thr Glu Lys Ser Leu Thr 330 325

Ile	Thr	Gly	Ile 340	Thr	Gly	Thr	Ile	Asp 345	Phe	Val	Ser	Asn	Ile 350	Ala	Thr
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	т1.	Th∽	Th∽	Dro	Pro	Lon	Wa l	Gly	Glu		Tle	Dhe	Ser	Glu	
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mb	N1-	T	61		Gly	C1	C1	Tlo		Thr	λαπ	Tuc	Ton		Lou
1111	Ald	гуу	420	птэ	Gry	(31 Å	GIY	425	Суз	1111	nsii	цуз	430	361	neu
Cor) cn	Lou		Thr	Val	Thr	T.all		Lve	λen	Ser	Δla		Glu	Ser
ser	ASII	435	гуs	1111	vaı	1111	440	1111	uys	ASII	361	445	Буз	314	SCI
C1	C111		т10	Dho	Thr	λen		Δla	Ser	Tle	Pro		Thr	Δsn	Thr
GIY	450	ліа	116	FIIC	1111	455	БСС	niu	DCI	1	460			тър	
Dro		cor	Car	Thr	Pro		Ser	Sar	Ser	Pro		Ser	Thr	Pro	Ghu
465	GIU	SEI	26.1	1111	470	361	JCI	JCI	DCI	475	niu	501	1111		480
	Wal.	λla	Sar	ΔΊα	Lys	Tle	Δen	Δra	Phe		Δla	Ser	Thr		
vaı	Vai	AIG	361	485	БүЗ	110	71511	y	490	- 110		001		495	0.0
Pro	λla	λla	Ero		Leu	Thr	Glu	Δla		Ser	Asn	Gln	Thr		Gln
rio	nia	AIG	500	JCI	Dea	****	OIG	505	010		р		510	····	
Thr	Glu	Thr		Asn	Thr	Asn	Ser		Tle	Asp	Val	Ser		Glu	Asn
1111	GIU	515	JCI	.nsp	11,1	711,11	520	ор		····		525		010	
Tlo	Leu		ī/a l	Δ1а	Ile	Agn		Asn	Thr	Ser	Ala		Lvs	Glv	Glv
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cccaataata gtggtaagga tgttctatct gatgtcttgc aaattgcttc gattcagatc
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aacaaaatga agtctagaaa agcttgtggt gtagctgttg gtgcaacgtt aatcgacgct
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His Ala Ser Gln Asp Asp Pro Leu Tyr Val Leu Gly Asn Ser Tyr Cys
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Tyr Gln Gly Asp Trp Thr Phe Ser Trp Lys Asp Ser Asp Glu Gly His
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                                     540
Ser Leu Ile Ala Asn Trp Thr Pro Lys Asn Tyr Val Pro His Pro Glu
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Arg Gln Ser Thr Leu Val Ala Asn Thr Leu Trp Asn Thr Tyr Ser Asp
            565 570 ·
Met Gln Ala Val Gln Ser Met Ile Asn Thr Thr Ala His Gly Gly Ala
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                          585
Tyr Leu Phe Gly Thr Trc Gly Ser Ala Val Ser Asn Leu Phe Tyr Val
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His Asp Ser Ser Gly Lys Pro Ile Asp Asn Trp His His Arg Ser Leu
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Gly Tyr Leu Phe Gly Ile Ser Thr His Ser Leu Asp Asp His Ser Phe
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                                 635
Cys Leu Ala Ala Gly Gln Leu Leu Gly Lys Ser Ser Asp Ser Phe Ile
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Thr Ser Thr Glu Thr Thr Ser Tyr Ile Ala Thr Val Gln Ala Gln Leu
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Ala Thr Ser Leu Met Lys Ile Ser Ala Gln Ala Cys Tyr Asn Glu Ser
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                                         685
Ile His Glu Leu Lys Thr Lys Tyr Arg Ser Phe Ser Lys Glu Gly Phe
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Gly Ser Trp His Ser Val Ala Val Ser Gly Glu Val Cys Ala Ser Ile
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Pro Ile Val Ser Asn Gly Ser Gly Leu Phe Ser Ser Phe Ser Ile Phe
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Ser Lys Leu Gln Gly Phe Ser Gly Thr Gln Asp Gly Phe Glu Glu Ser
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Ser Gly Glu Ile Arg Ser Phe Ser Ala Ser Ser Phe Arg Asn Ile Ser
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Leu Pro Ile Gly Ile Thr Phe Glu Lys Lys Ser Gln Lys Thr Arg Thr
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Tyr Tyr Tyr Phe Leu Gly Ala Tyr Ile Gln Asp Leu Lys Arg Asp Val
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Glu Ser Gly Pro Val Val Leu Leu Lys Asn Ala Val Ser Trp Asp Ala
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             805
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Gln Arg Ala Leu His Arg Leu Gln Thr Leu Leu Asn Val Ser Cys Val
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Arg Phe
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		2.5	Leu	Thr			40					43			
	- 0	Thr		Val		55					60				
	Asn			Ala	70					10					00
Gly				Val 85					90					23	
			100	Thr				105					LIU		
		115		Ile			120					123			
	120			Ala		135					140				
1 A E				Thr	150					122					100
				Leu 165					170					1/3	
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		105		Ser			200					203			
	210			Ala		215					220				
225				Ile	つてり					235)				240
				Ala 245					251	,				233	
			260	Asp				265	•				2/0	,	
		27	_				280)				285	•		Tyr
	200	`				295	5				300	,			Asn Gln
201	-				210	1				31:)				Gln 320 Cvs
				325					33	U				33.	
			34	n				34	5				331	,	· Phe
		2.5	_				361	n				30:	כ		y Lys
	27	Λ				37	5				301	,			y Pro
		n Ph	e Le	u Arç	asr 390		e Al	a AS	II AS	29 39	y 01) 5	, 111		7	r Leu 400
	y Gl			405	ι Leι	Se د			41	a As	р Ту			41.	
			12	n Lei	ı Lys			42	5				7.7	•	p Val
As	n Gl	y Va	al Th	r Va	l Se	r Se	r Gl	n Al	a Il	e Se	r Me	t Gl	y Se	r Gl	y Gly

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			820					825					830	Met	
		835					840					845		Asn	
	850					855					860			Thr	
Ser 865	Lys	Leu	Tyr	Leu	Asn 870	Glu	Leu	Arg	Pro	Phe 875	Val	Gln	Ala	Glu	Phe 880

Ser Tyr Ala Asp His Glu Ser Phe Thr Glu Glu Gly Asp Gln Ala Arg 890 Ala Phe Lys Ser Gly His Leu Leu Asn Leu Ser Val Pro Val Gly Val 905 Lys Phe Asp Arg Cys Ser Ser Thr His Pro Asn Lys Tyr Ser Phe Met 920 Ala Ala Tyr Ile Cys Asp Ala Tyr Arg Thr Ile Ser Gly Thr Glu Thr 940 935 Thr Leu Leu Ser His Gln Glu Thr Trp Thr Thr Asp Ala Phe His Leu 955 950 Ala Arg His Gly Val Val Val Arg Gly Ser Met Tyr Ala Ser Leu Thr 970 965 Ser Asn Ile Glu Val Tyr Gly His Gly Arg Tyr Glu Tyr Arg Asp Ala 985 Ser Arg Gly Tyr Gly Leu Ser Ala Gly Ser Lys Val Arg Phe 1000 <210> 191 <211> 977 <212> PRT <213> Chlamydia <400> 191 Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Ser Ser Leu 1 5 Val Pro Ser Ser Asp Pro His His His His His Gly Leu Ala Arg 25 Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val Pro Asp Pro 40 Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly Asp Thr His 55 Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile Leu Ala Ile 75 70 Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile Thr Asp Tyr 90 85 Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe Ala Lys Asn 105 Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser Pro Asn Ser 125 120 Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile Phe Glu Asn 140 135 Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr Ala Ala Asp 155 150 Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu Tyr Ile Asn 170 His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser Tyr Val Gln 185 190 Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser Glu Asn Gln 205 200 195 Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr Asn Thr Ala 220 215 Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser Phe Glu Ser 235 230 Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys Ala Gly Gly 250 245

Ala	Ile	Phe	Ser 260	Pro	Ile	Cys	Ser	Leu 265	Thr	Gly	Asn	Arg	Gly 270	Asn	Ile
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Glu	Ala 290	Ser	Asp	Gly	Gly	Ala 295	Ile	Lys	Val	Thr	Thr 300	Arg	Leu	Asp	Val
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			660					665					His 670		
		675					680					685	Ala		
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            725
Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe Ser Gln Thr
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Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val Ser Ser Lys
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Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln Glu Gly Phe
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Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp His Asn Cys
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785
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Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu Pro Met Lys
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         820 825
Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu Gly Ala Leu
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 Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly Ala Tyr Pro
  - <sub>850</sub> - <sub>855</sub>
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 865 870
                                875
 Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro Gln Ala Trp
            885 890
 Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln Glu Pro Gly
          900 905
 Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe Gly Ser Gly
       915 920
 Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser Gln Gln Thr
   930 935 940
 Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His Gly Phe Tyr
 945 950 955
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             965
  Phe
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  Glu Val Glu Pro Ala Pro Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe
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  Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser
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Asn	Phe 130	Ser	Asp	Ile	Tyr	Gly 135	Gly	Ala	Ile	Phe	Thr 140	Gly	Ser	Leu	Arg
Glu 145	Glu	Asp	Lys	Leu	Asp 150	Gly	Gln	Ile	Pro	Glu 155	Val	Leu	Ile	Ser	Gly 160
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His	Leu	Pro	His 180	Thr	Gly	Gly	Gly	Ala 185	Ile	Cys	Thr	Gln	Asn 190	Leu	Thr
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225			_		230			_	_	235			Phe		240
				245					250				His	255	
			260					265					Asp 270		
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				325					330				Lys	335	
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_		355					360					365	Pro		
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385					390					395			His		400
				405					410				Gly	415	
			420					425					Arg 430		
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Asp	Leu	Gln	Ile	His 485	Val	Ala	Thr	Pro	Glu 490	Ile	Glu	Glu	Asp	Thr 495	Tyr
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Val	Ile	Asn 515	Trp	Asn	Pro	Thr	Gly 520	Tyr	Arg	Leu	Asp	Pro 525	Gln	Lys	Ala

PCT/US99/29012

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Phe 65	Gln	Glu	Lys	Asp	Ala 70	Asp	Thr	Leu	Pro	Gly 75	Lys	Val	Glu	Gln	Ser 80
Thr	Leu	Phe	Ser	Val 85	Thr	Asn	Pro	Val	Val 90	Phe	Gln	Gly	Val	Asp 95	Gln
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Asn	Leu	Asp 115	Ser	Pro	Arg	Asp	Gly 120	Glu	Ser	Phe	Leu	Gly 125	Ile	Ala	Phe
Val	Gly 130	Asp	Ser	Ser	Lys	Ala 135	Gly	Ile	Thr	Leu	Thr 140	Asp	Val	Lys	Ala
Ser 145	Leu	Ser	Gly	Ala	Ala 150	Leu	Tyr	Ser	Thr	Glu 155	Asp	Leu	Ile	Phe	Glu 160
Lys	Ile	Lys	Gly	Gly 165	Leu	Glu	Phe	Ala	Ser 170	Cys	Ser	Ser	Leu	Glu 175	Gln
Gly	Gly	Ala	Cys 180	Ala	Ala	Gln	Ser	Ile 185	Leu	Ile	His	Asp	Cys 190	Gln	Gly
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	210	_			_	215	_	_	_		220			Thr	_
225			_		230			-		235			_	Met	240
				245					250					Ala 255	
			260	_				265		_	_		270	Phe	
		275	_	_			280			•		285		Ser	_
_	290					295	_				300		_	Ala	
305				_	310					315				Gly	320
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	370 Glu					375					380			Phe	
385					390					395					400
				405					410					Phe 415	
		-	420					425			_		430	Lys	
		435					440					445		Asp	
	450					455					460			Asn	
Ser 465	Leu	Ser	Glu	Asn	Ala 470	Gly	Val	Leu	Thr	Phe 475	Lys	Asp	Asn	Ile	Val 480
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Ala	Thr	Gly	Lys	Val	Glu	Ile	Thr	Asn	Asn	Ser	Gly	Gly	Ile	Ser	Phe

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Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser
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Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala
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Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala
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Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His Gly Met Asp Ser
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Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly
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	450					455					460	Gly			
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Ala	Ala	Thr 515	Ala	Thr	Pro	Thr	Thr 520	Thr	Pro	Thr	Ala	Thr 525	Thr	Thr	Thr
Ser	Asn 530	Gln	Val	Ile	Leu	Gly 535	Gly	Glu	Ile	Lys	Leu 540	Ile	Asp	Pro	Asn

Gly T					ちちん					コココ					500
545 Leu I				Pro	Thr				570					3,3	
Val I			EOA	Asp				วชว					330		
Thr I		-0-	Pro				600					000			
Lys 1	~ · · ·	Asp				615					020				
Phe '	Tyr	Ala	Asn	Ser	Ile	Leu	Gly	Ser	Gln	Met	Ser	Met	Val	Thr	Val 640
					630					633					0.10
Lys (Gln	Gly	Leu	Leu 645	Asn	Asp	Lys	мет	650	ьеи	Ala	нтЭ	FIIC	655	010
Val			cco	Asn				665	Gly				0,0		
Gln		/ D F	Thr				680	Glu				002			
		Val				695	Lys				700				
Ala	Ala				710		Gly			710					
Asn				725			Ser		/30					, 55	
			740				Phe	745					, 50		
		255					Gly 760	1				700			
	770					775	5				760				Gly
Glu	Trp	Glu	Asp	Leu			Leu	Thr	Ala	Leu 795	Arg	Val	. Ser	Ser	800
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Ile	e G1 93		a As	p Al	a Hi	s Th 93	r Le	u Ala	a Hi	s Me	t Me	C AS	п Су:	s 61)	y Ala
Arc 945		t Th	r Ph	е											
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<212> PRT <213> Chlamydia

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Ser Glu Asn Thr Ala Lys Gly His Gly Gly Gly Ile Cys Thr Asn Lys

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Leu S	_		_	405	T	Tvc	ሞክ	r V	'al '	410 Thr	Leu	Thr	Lys			Ala
Lys G	1 (cor	420 Glv	Glv	Ala	Ile	Ph	e T	hr	Asp	Leu	Ala	Ser	Ile	Pro	Thr
Thr A	sp 1	Thr	Pro	Glu	Ser	Ser	Th	r I	ro	Ser	Ser	Ser	Ser	Pro	Ala	Ser
						166						400				
4 Thr P	ro	Glu	Val	Val	Ala	Ser	Al	a I	уys	Ile	Asn	Arg	Pne	Pne	Ala	480
465 Thr A	la	Glu	Pro			Pro) Se	er l	Leu	490	Giu	AIG	010	-00	495	
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Ile	2311	Δen	300	Leu	Ası	ı Va	A	l.a	Ile	Asn	Gln	Asn	Thr	Ser	Ala	Lys
							-	<i>)</i> ()					227			
Lys (Gly	Gly	Ala	Ile	Туз	G1;	y Ly	ys	Lys	Ala	Lys	Leu	Ser	Arg	H	ASII
Asn I	Leu	Glu	Leu	Ser	Gly	/ As	n S	er	Ser	GIn	555	Val	GIY	Gry	Ory	560
545 Cys :																
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Val	Thr	Leu	Sei	ASI	n Le	u Ly	s S	er	Thr	Phe	Thr	Phe	Ala	Asp	Asn	Thr
Val	Lys	Ala	a Il	e Va	1 G1	Se גי	r T	hr	Pro	Glu	ı Ala	620	Glu	GIU	. 110	Pro
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Ser	Asp	Th	r Gl	y As	n Al	.a G	lu s	Ser	Gly	/ Gl	u GI	п Ьег	689 1 GII	ı AS	361	Thr
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705 Asn	Gli	ı Se	r Va	al Se	er S	er S	er	Ser	Ly	s Se	r Gl	y Se	r Se	r Th	r Pro	o Gln 5
Asp	Gly	, Gl	y A	la A	la S	er S	er	Gly	Al	a Pr	o Se	r Gl	y As	p GI:	n se:	r Ile
Ser	Ala	a As	n A	la C	ys L	eu A	1a	Lys	se	г ту	r Al	a MI	a se 76	5		p Ser
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			8	20												

<211> 525

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Trp Gln Val Gly Leu Ala Leu Ser Tyr Arg Leu Asn Met Leu Val Pro
                                 410
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Tyr Ile Gly Val Asn Trp Ser Arg Ala Thr Phe Asp Ala Asp Thr Ile
                                               430
                             425
           420
Arg Ile Ala Gln Pro Lys Leu Lys Ser Glu Ile Leu Asn Ile Thr Thr
                                  445
             440
Trp Asn Pro Ser Leu Ile Gly Ser Thr Thr Ala Leu Pro Asn Asn Ser
            455
Gly Lys Asp Val Leu Ser Asp Val Leu Gln Ile Ala Ser Ile Gln Ile
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465 470
Asn Lys Met Lys Ser Arg Lys Ala Cys Gly Val Ala Val Gly Ala Thr
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Asn Glu Arg Ala Ala His Met Asn Ala Gln Phe Arg Phe
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Pro Met Pro Arg
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Val Trp Glu Tyr
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Lys Lys His Asn
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Ser Lys His Ile Val Lys
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Val Glu Ile Thr Gln Ala Val Pro Lys Tyr Ala Thr Val Gly Ser Pro
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Tyr Pro Val Glu
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Thr Thr Pro Thr
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Asp Gly Lys Leu
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Lys Ser Lys Ile
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Pro Glu Gly Ser
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His Ala Val Ile
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Arg Ser Ile Asp
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Glu Leu Arg Ile
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attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                       180
gegggetett cegcacacat tacagettee caagtgteea aaggattagg ggatgegaga
                                                                       240
actgttgteg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
                                                                       300
caaagettet teteteacat gaaagetget agteagaaaa egeaagaagg ggatgagggg
                                                                        360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                        420
ateggaggaa ttacctacct egegacatte ggagetatee gteegattet gtttgtcaac
                                                                        480
aaaatgctgg caaaaccgtt tctttcttcc caaactaaag caaatatggg atcttctgtt
                                                                        540
agctatatta tggcggctaa ccatgcagcg tctgtggtgg gtgctggact cgctatcagt
                                                                        600
 gcgnaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                        660 ·
 gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                        729
 ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
                                                                        780
 gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct
                                                                        840
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                                                                        897
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                                  25
 Lys Thr Lys Gly Val Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
                                                  45
                              40
 Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
                                              60
                          55
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60

WO 00/34483 126

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Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                    70
                                        75
Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
                85
                                    90
Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
                               105
Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                            120
                                               125
His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
                        135
Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                   150
                                       155
Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                                   170
                165
                                                        175
Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
                               185
            180
Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala
                            200
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly
                        215
Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
                                        235
                    230
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                                    250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
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Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
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Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala
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840

897

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gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct
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                              25
Lys Thr Lys Gly Met Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
                          40
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
                       55
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                                      75
                   70
Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
                                   90
Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
                              105
Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                          120
His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Tie Gly Gly Ile
                                          140
                      135
 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                   150
                                      155
 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                165
                                  170
 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
                               185
 Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala
                                              205
                            200
 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly
                                          220
                        215
 Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
                                       235
                    230
 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                                    250
                245
 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
                                265
 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
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                                                                       120
gttccttacg ttcagagaag gattttgtcg cgttagttgg taaagtttta gctgataacg
                                                                       180
tagttgatgc ggattcttca ttagtttacg ggaaagctgg agagaagcta agtactgcta
                                                                       240
tgctaaaacg catcttagat acgggagtcc aatctttgaa gattgctgtt ggcgcagatg
                                                                       300
aaaatcaccc aattattaag atgctcgcaa aagatcctac ggattcttac gaagctgctc
                                                                       360
ttaaaqattt ttatcgcaqa ttacqaccaq gaqaqcctqc aactttaqct aatqctcqat
                                                                       420
ccacaattat gcgtttattc ttcgatgcta aacgttataa tttaggccgc gttggacgtt
                                                                       480
ataaattaaa taaaaaatta ggcttcccat tagacgacga aacattatct caagtgactt
                                                                       540
tgagaaaaya agatgttatc ggcgcgttga aatatttgat tcgtttgcga atgggcgatg
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agaagacate tategatgat attgaceatt tggcaaaceg aegagttege tetgttggag
                                                                       660
aactaattca gaatcactgt
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<213> Chlamydia
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                                                                      120
agetectaac aaagagetaa tttttteete tteettgttt ttettgaggeg etgtggaete
                                                                      180
taaatatago aagtgotott ggaacacoto atoaacaato gottgtoota gattaggtat
                                                                       240
agagactgtc tctccatcaa ttaaatggag tttcaaagta atatcccctt ccgtccctcc
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atcacaagac totatgaaag ctatotgatt coatogagca gazatgtatg gggaaatac
                                                                      359
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ggaataacaa gaaataggta atcggtacca ttgatagaac gaacacgaca aatcgcagaa
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ggtt
                                                                      124
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ataagagcac ggatacgctt atagtggtta tagacggcaa ccgaaatcgt tttttcgcg
                                                                      120
cgctcttgtc caatgacata agagtcgatg tggcgtttga tttctttagg ggttaacact
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                                                                       120
tgttatcgat agcttggttc ccagagaact gacaagtccc gctacattga gagaatgtaa
                                                                       180
cctgttctcc atagatagct cctcctacta cacctgaata agttggtgtt gctggagatg
                                                                       240
atggtgcggc tgctgcggct gcttgtaggg aagcagcagc tgcagcaggt gctgaagctg
                                                                        300
ttgttgcgac tcctgtggat gaggagtttg ctttgttgtt cgagaaagag aagcctgatt
                                                                        360
tcagattaga aatatttaca gttttagcat gtaagcctcc accttctttc ccaacaaggt
                                                                        420
tetetgttac agataaggag actagangca tetagtttta aagatttttt acagcagata
                                                                        480
                                                                        511
cctccaccta tctctgtagc ggagttctca g
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<211> 598
<212> DNA
<213> Chlamydia
 <400> 272
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                                                                        120
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                                                                        240
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                                                                        300
 agacaacatc accctatcta atttgacagg gaagactcta ttccaagaga atactgccaa
                                                                        360
 aaaagagggc ggtggactct tcataaaagg tacagataaa gctcttacaa tgacaggact
                                                                        420
 ggatagtttc tgtttaatta ataacacatc agaaaaaacat ggtggtggga gcctttgtta
                                                                        480
                                                                        540
 ccaaagaaat ctctcagact tacacctctt gatgtggaaa caattccagg aatcacgcct
 gtacatggtg aaacagtcat tactggcaat aaatctacag gaggtaatgg tggagggc
                                                                        598
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 <211> 126
 <212> DNA
 <213> Chlamydia
  <400> 273
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  gatagetttt tattageegt tittageate etaatgagat eteetegtte gtaacaaata
                                                                         120
                                                                         126
  cgagag
  <210> 274
  <211> 264
  <212> DNA
  <213> Chlamydia
  <400> 274
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  tttttcaaaa aagaatttaa acattaattg ttgtaaaaaa acaatattta ttctaaaata
                                                                         120
  ataaccatag ttacggggga atctctttca tggtttattt tagagctcat caacctaggc
                                                                         180
  atacgcctaa aacatttcct ttgaaagttc accattcgtt ctccgataag catcctcaaa
                                                                         240
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<212> DNA
<213> Chlamydia
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                                                                       120
ttaaaacttg ttctcttaaa ttaattctag tatttaagta ttcaacatag cccattatta
                                                                       180
attgaattgg ataattttgc cttaataatt cacattcttt ttcagtaatt ttaggttcta
                                                                       240
aaccqtaccq ctttttttct aaaattaatq tttcttcatt attcatttta taaqccactt
                                                                       300
tcctttattt tttgattttg ttcttctgtt agtaatgctt caataatagt taataattt
                                                                       359
<210> 276
<211> 357
<212> DNA
<213> Chlamydia
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                                                                        60
atgggtagta gtgactctaa cgttttttat tattaagacg atccccggag atccttttaa
                                                                       120
tgatgaaaac ggaaacatcc tttcgccaga aactttagca ctattaaaga atcgttacgg
                                                                       180
gttagataag cctttattca cccagtatct tatctatttg aaatgtctgc taacactaga
                                                                       240
tttcggggaa tctcttatct acaaagatcg aaatctcagc attattgctg ccgctcttcc
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atottcogot attottggac ttgaaagott gtgtttactc gtgccgaatt cggatcc
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<211> 505
<212> DNA
<213> Chlamydia
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agcactaaaa gagactcctc ttcaagaacg agagtgtaag cagggtgagg aggaacttca
                                                                       120
ggtaaaaatc ctaaggccat accaggatgc gacaggaaag agatatctcc attaggagct
                                                                      180
cggagacacg ctgggttgtg gccacaagaa tagtattcta gttctcgtgt tgcgtaatga
                                                                       240
taacaataaa tgcatagtgt tacaaacatc ccagattcag ctgtctgttg atagaagaga
                                                                       300
gcagctgttt gttgaacggc ttcttgaata gaggagagct cactcaaaaa ggtatgtaac
                                                                       360
atgtttttca ggaataagga gtaggcgcac gcattgactc ctttcccgga agcatcagca
                                                                       420
acgattagaa agagtttagc ttggggacct tcgcctataa caaagatatc aaagaaatct
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<211> 407
<212> DNA
<213> Chlamydia
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                                                                      120
ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat
                                                                      180
ccttcgccca attacagaga cacagcttca ggcctttatg gacgtctggt ctcttctaga
                                                                      240
aacaaatage teetatetgt eeccagagag egtgettaeg geecetaete etteaagtag
                                                                      300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat
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<211> 577 <212> DNA <213> Chlamy	<i>r</i> dia					
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<210> 282 <211> 607 <212> DNA <213> Chlamy	⁄dia					
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132

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gctgaaaaaa cctaaattca aaagaatgac tcgccgctca tcttcagaaa gacgatccga	240
cttccataat tcgatgtctt tccccatggg gatctctgta gggagccagt tatttgcgca	300
gccattcaaa taatgttccc aagcccattt gtacttaata ggaacaagtt ggttgacatc	360
gacctggttg cagttcacta gacgcttgct atttagatta acgcgtttct gttttccatc	420
taaaatatct gcttgcataa gaaccgttaa ttttattgtt aatttatatg attaattact	480
gacatgette acaccettet tecaaagaac agacaggtge tttetteget ettteaacaa	540
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Cacciac	807
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ttctagtaag caggaaaaaa gctcgtaacg cctcttcatc ggtggctaat gtataaaagg	180
ctcgtcctga ctcatgcatt tcggcatgat ctggcccaac tgaaggataa tctaatccag	. 240
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atccgtggaa tactccaggt cgccctgttg caaaacgtgc tgcatgtttt cctgaagaaa	. 360
tgcccagtcc tcccccttcc actccaatta attggacttt tggattcggg ataaaatgat	429
ggaaaaatcc aatagcgttg gagccacctc cgatacatgc aatcagaata tcaggatctc	480
ttcctgcaac tgcatggatt tgctctttca cttcagcgct tataacagac tgaaaaaatc	540
gaacgatatc gggataaggt aaaggtccta aggccgatcc taagcaatag tgagtaaatg	600
agtgtgttgt tgcccaatct tgtagagctt gattaactgc atctttgagt ccacaagatc	. 660
cttttgttac agaaacgact tcagcaccta aaaagcgcat tttctctaca tttggtttct	720
gtcgttccac atcttttgct cccatgtata ctacacaatc taatcctaga taagcacacg	780
ctgttgctgt tgctactcca tgttgtcccg cacctgtttc agctacaaca cgtgtttcc	840
caagatattt agcaagcaaa cactgaccaa gagcattatt cagtttatgt gctcctgtat	900
gcaaaagatc ttcgcgttta agaaatactc tagggccatc aatagctcga gcaaaattct	960
taacttcagt cagaggagtt tgtctccccg catagttttt caaaatacaa tctagttcag	1020
ataaaaaact ttgctgagtt ttgagaatct cccattccgc ttttagattc tgtatag	1077
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ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat	180
ccttcgccca attacagaga cacagcttca ggcctttatg gacgtctggt ctcttctaga	240
aacaaatagc tcctatctgt ccccagagag cgtgcttacg gcccctactc cttcaagtag	300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat	360
ccgtatgaga aaataggatt agggaaacaa aacgacagca aaccaca	407
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212. DNA	

<212> DNA

<213> Chlamydia

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                                                                       120
tgggaaaaaa gaatctgctg aattcgaaaa gatgaaaaac caattctcta acagcatggg
                                                                       180
gaagatggag gaagaactgt cttctatcta ttccaagctc caagacgacg attacatgga
                                                                       240
aggtctatcc gagaccgcag ctgccgaatt aagaaaaaaa ttcgaagatc tatctgcaga
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atacaacaca gctcaagggc agtattacca aatattaaac caaagtaatc tcaagcgcat
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gcaaaagatt atggaagaag tgaaaaaagc ttctgaaact gtgcgtattc aagaaggctt
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gtcagtcctt cttaacgaag atattgtctt atctatcgat agttcggcag ataaaaccga
                                                                        480
tgctgttatt aaagttcttg atgattcttt tcaaaataat taacatgcga agctagccga
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ggagtgccgt atgtctcaat ccacttattc tcttgaacaa ttagctgatt ttttgaaagt
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cgagtttcaa ggaaatggag ctactcttct ttccggagtt gaagagtcg aggaagcaaa
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aacggcacac atcacattct tagataatga aaaatatgct aaacatttaa aatcatcgga
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                                                                        120
 attgcaaccg cacgcgattg aatgatacgc aagccatttc catcatygaa aagaaccett
                                                                        180
 ggacaaaaat acaaaggagg ttcactccta accagaaaaa gggagagtta gtttccatgg
                                                                        240
 gttttcctta tatacacccg tttcacacaa ttaggagccg cgtctagtat ttggaataca
                                                                        300
 aattgtcccc aagcgaattt tgttcctgtt tcagggattt ctcctaattg tretgtcagc
                                                                        350
 catccgccta tggtaacgca attagctgta gtaggaagat caactccaaa caggtcatag
                                                                        420
 aaatcagaaa gctcataggt gcctgcagca ataacaacat tcttgtctga gtgagcgaat
                                                                        480
 tgtttaaaag atgggcgatt atgagctacc tcatcagaga ctattttaaa tagatcattt
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 tgggtaatca atcettetat agacceatat teatcaatga taateteg
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 <213> Chlamydia
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  \langle 223 \rangle n = A,T,C or G
  <400> 287
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  acaagtaget gttatgtatg gttetagttg ettactgege geegtgggeg atttagegaa
                                                                         120
  aaatgattet tetatteaag taegeateae tgettategt getgeageeg tgttggagat
                                                                         180
  acaagatett gtgeeteatt tacgagttgt agteeaaaat acacaattag atggaaegga
                                                                         240
  aagaagagaa gcttggagat ctttatgtgt tcttactcgg cctcatagtg gtgtattaac
                                                                         300
  tggcatagat caagctttaa tgacctgtga gatgttaaag gaatatcctg aaaagtgtac
                                                                         360
  ggaagaacag attcgtacat tattggctgc agatcatcca gaagtgcagg tagctacttt
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134

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allo, chiamyara	
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Coogugugug C	171
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tgcctgcaga tgtttatgcg cgttttcaga gactacaagg caaagaggtt ttgtatattt	180
gtggttctga tgaatacgga atcgcaatta cccttaatgc agagttggca ggcatggggt	240 300
atcaagaata tgtcgacatg tatcataagc ttcataaaga taccttcaag aaattgggaa tttctgtaga tttcttttcc agaactacga acgcttatca tcctgctatt gtgcaagatt	360
tctatcgaaa cttgcaggaa cgcggactgg tagagaatca ggtgaccgaa cagctgtatt	420
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tttgatgtaa attagcgcaa ttagaggggg atgaggttac ttggaaatat aaggagcgaa	180
gcgatgaagg agatgtattt gctctggaag caaaggtttc tgaagctaac agaacattgc	240:
gtcctccaac aatcgcctga ggattctggc tcatcagttg atgctttgcc tgaatgagag	300
cggacttaag tttcccatca gagggagcta tttgaattag ataatcaaga gctagatcct ttattgtggg atcagaaaat ttacttgtga gcgcatcgag aatttcgtca gaagaagaat	360 420
Catcatcgaa cgaattitte aateetegaa aatettetee agagaetteg gaaagatett	480
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aaatgcttta agctaagtaa ggcggtttct gactgtgtcg taggatcgct ggaagaggcg	240
ggatgcacag gggacgcatt gacctccgcg agaaacgccc agggtatgtt aaaaacaact	300
cgagaagttg ttgccttagc taatgtgctc aatggagctg ttccatctat cgttaactcg	360
actcagaggt gttaccaata cacacgtcaa gccttcgagt taggaagcaa gacaaaagaa	420

agaaaaaa gcttccag ttacgtcc gtaggcaa gctggtgc ctatacaa ctaagcga actcttc attacag ggcttat <210> 2 <211> 3 <212> F	ggg aa cgt ta att ti ctg ti atg ag cgg a ttg a ttg c ata g	agett aatgt tggca tggcg gagat aaggg aaaag ttgct	gtac taat cggc gaat gtgc catt	ggca caa tgt cgc ctt acg aga	taaa tgct atta agaa taaa gttg	ggt ctc gga gaa aac gaa gta	gcaa acag atta caaa caac gtgg ggag	caaa tgac agct aatc sttgc gatgg	ac c ca t gt t tc a ta c ag t	taat caaa gttg ctcta caaa	ttag cata cgtg agtg aaaa	a ca t gg c ga g gg ig aa	aago aagga aagga aacgt aatgt	gact gagtt aatcc gatt ttta caccg	480 540 600 660 720 780 840 900 960 1002
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<400> 2 Met Ala	92 Thr	Asn I	Ala :	Ile A	Arg S	Ser A	Ala	Gly :	Ser .	Ala	Ala :	Ser	Lys 15	Met	
1 Leu Leu															
Lys Gly		Tyr	Cys	Tle	Gln	Gln 40	Phe	Phe	Thr	Asn	Pro 45	GIY	ASII	пуъ	
Leu Ala	35 a Lys	Phe	Val	Gly .	Ala	Thr	Lys	Ser	Leu	Asp	Lys	Cys	Phe	Lys	
50 Leu Se					C E					00					
65 Gly Cy	s Thr	Gly	Asp	Ala	Leu	Thr	Ser	Ala 90	Arg	Asn	Ala	GIn	95	мес	
Leu Ly	s Thr	Thr	85 Arq	Glu	Val	Val	Ala	Leu	Ala	Asn	Val	Leu	Asn	Gly	
							1117								
Ala Va						7 7 11					127				
Arg Gl	n Ala	Phe	Glu	Ľeu	Gly	Ser	Lys	Thr	Lys	Glu 140	Arg	Lys	Thr	Pro	
13 Gly Gl	30	· Sar	īvs	Met	135 Leu	Leu	Thr	Arg	Gly	Asp	Tyr	Leu	Leu	Ala	
145 Ala Se															
Thr Pl	ne Gly	, Val	165 Leu	Arg	Pro	Leu	Met	Leu	Ile	Asn	Lys	Leu	Thr	Ala	
Lys P															
Ala G	ly Il	e Met	Thr	Ile	Asn	His	Met	Ala	Gly	Val 220	Ala	Gly	Ala	vaı	
Leu T															
Gly A	sp Va	l Ile	245 Leu	Ser	Ala	Glu	Arg	Ala	Lev	ı Arg	Lys	Glu	His	val	
Ala T		~ ~ ~					/n-	١.							
	al Va	l Ası	o Gly	/ Val	Lys	Leu	ılle	Pro	Leu) Pro 300) Ile)	Thi	va:	r WIG	
2	90				295	,									

136

WO 00/34483 PCT/US99/29012

7

Cys Ser Ala Ala Ile Ser Gly Ala Leu Thr Ala Ala Ser Ala Gly Ile 305 $310 \hspace{1.5cm} 315 \hspace{1.5cm} 320$ Gly Leu Tyr Ser Ile Trp Gln Lys Thr Lys Ser Gly Lys

325

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<211> 7

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<213> Chlamydia

<400> 293

tgcaatc

<210> 294

<211> 196

<212> PRT

<213> Chlamydia

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5 10 15

Ala Val Val Cys Gly Glu Glu Lys Glu Ile Ser Leu Ala Asp Phe Arg
20 25 30

Gly Lys Tyr Val Val Leu Phe Phe Tyr Pro Lys Asp Phe Thr Tyr Val 35 40 45

Cys Pro Thr Glu Leu His Ala Phe Gln Asp Arg Leu Val Asp Phe Glu 50 55 60

Glu His Gly Ala Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr
65 70 75 80

His Ser Arg Trp Leu Thr Val Ala Arg Asp Ala Gly Gly Ile Glu Gly

Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala 100 105 110

Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe 115 120 125

Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu 130 $$135\ \ \, 140\ \ \, 135$

Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu 145 150 155 160

Ile Phe Phe Glu Asn His Gly Met Val Cys Pro Ala Asn Trp Arg Ser 165 170 175

Gly Glu Arg Gly Met Val Pro Ser Glu Glu Gly Leu Lys Glu Tyr Phe 180 185 190

Gln Thr Met Asp 195

<210> 295

<211> 181

<212> PRT

<213> Chlamydia

<400> 295

Lys Gly Gly Lys Met Ser Thr Thr Ile Ser Gly Asp Ala Ser Ser Leu

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Thr Lys Gly Asn Thr Cys Ser Lys Ile Leu Asp Ile Ala Leu Ala Ile

Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys 55

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile 70

Gly Ser Ala Cys Val Gly Ala Gly Ile Ser Arg Leu Met Tyr Arg Ser

Ser Tyr Ala Ser Leu Glu Ala Lys Asn Val Leu Ala Glu Gln Arg Leu 105

Arg Asn Leu Ser Glu Glu Lys Asp Ala Leu Ala Ser Val Ser Phe Ile 120

Asn Lys Met Phe Leu Arg Gly Leu Thr Asp Asp Leu Gln Ala Leu Glu 130

Ala Lys Val Met Glu Phe Glu Ile Asp Cys Leu Asp Arg Leu Glu Lys 155 150 145

Asn Glu Gln Ala Leu Leu Ser Asp Val Arg Leu Val Leu Ser Ser Tyr

Thr Arg Trp Leu Asp 180

<210> 296

<211> 124

<212> PRT

<213> Chlamydia

<400> 296

Ile Tyr Glu Val Met Asn Met Asp Leu Glu Thr Arg Arg Ser Phe Ala

Val Gln Gln Gly His Tyr Gln Asp Pro Arg Ala Ser Asp Tyr Asp Leu 25

Pro Arg Ala Ser Asp Tyr Asp Leu Pro Arg Ser Pro Tyr Pro Thr Pro

Pro Leu Pro Ser Arg Tyr Gln Leu Gln Asn Met Asp Val Glu Ala Gly 55

Phe Arg Glu Ala Val Tyr Ala Ser Phe Val Ala Gly Met Tyr Asn Tyr

Val Val Thr Gln Pro Gln Glu Arg Ile Pro Asn Ser Gln Gln Val Glu

Gly Ile Leu Arg Asp Met Leu Thr Asn Gly Ser Gln Thr Phe Ser Asn

Leu Met Gln Arg Trp Asp Arg Glu Val Asp Arg Glu 120

<210> 297

<211> 488

<212> PRT

<213> Chlamydia

<400> 297

Lys Gly Ser Leu Pro Ile Leu Gly Pro Phe Leu Asn Gly Lys Met Gly

Phe Trp Arg Thr Ser Ile Met Lys Met Asn Arg Ile Trp Leu Leu Leu

Leu Thr Phe Ser Ser Ala Ile His Ser Pro Val Arg Gly Glu Ser Leu 40

Val Cys Lys Asn Ala Leu Gln Asp Leu Ser Phe Leu Glu His Leu Leu

Gln Val Lys Tyr Ala Pro Lys Thr Trp Lys Glu Gln Tyr Leu Gly Trp

Asp Leu Val Gln Ser Ser Val Ser Ala Gln Gln Lys Leu Arg Thr Gln

Glu Asn Pro Ser Thr Ser Phe Cys Gln Gln Val Leu Ala Asp Phe Ile 105

Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu

Ser Ala Tyr Leu Pro Tyr Thr Val Gln Lys Ser Ser Asp Gly Arg Phe 130 135

Tyr Phe Val Asp Ile Met Thr Phe Ser Ser Glu Ile Arg Val Gly Asp 150

Glu Leu Leu Glu Val Asp Gly Ala Pro Val Gln Asp Val Leu Ala Thr 165

Leu Tyr Gly Ser Asn His Lys Gly Thr Ala Ala Glu Glu Ser Ala Ala

Leu Arg Thr Leu Phe Ser Arg Met Ala Ser Leu Gly His Lys Val Pro 200

Ser Gly Arg Thr Thr Leu Lys Ile Arg Arg Pro Phe Gly Thr Thr Arg 215

Glu Val Arg Val Lys Trp Arg Tyr Val Pro Glu Gly Val Gly Asp Leu

Ala Thr Ile Ala Pro Ser Ile Arg Ala Pro Gln Leu Gln Lys Ser Met

Arg Ser Phe Phe Pro Lys Lys Asp Asp Ala Phe His Arg Ser Ser Ser 265

Leu Phe Tyr Ser Pro Met Val Pro His Phe Trp Ala Glu Leu Arg Asn 280

His Tyr Ala Thr Ser Gly Leu Lys Ser Gly Tyr Asn Ile Gly Ser Thr 290

Asp Gly Phe Leu Pro Val Ile Gly Pro Val Ile Trp Glu Ser Glu Gly 310

Leu Phe Arg Ala Tyr Ile Ser Ser Val Thr Asp Gly Asp Gly Lys Ser

His Lys Val Gly Phe Leu Arg Ile Pro Thr Tyr Ser Trp Gln Asp Met 345

Glu Asp Phe Asp Pro Ser Gly Pro Pro Pro Trp Glu Glu Phe Ala Lys 360

Ile Ile Gln Val Phe Ser Ser Asn Thr Glu Ala Leu Ile Ile Asp Gln 375

Thr Asn Asn Pro Gly Gly Ser Val Leu Tyr Leu Tyr Ala Leu Leu Ser 390

Met Leu Thr Asp Arg Pro Leu Glu Leu Pro Lys His Arg Met Ile Leu

Thr Gln Asp Glu Val Val Asp Ala Leu Asp Trp Leu Thr Leu Leu Glu 425

Asn Val Asp Thr Asn Val Glu Ser Arg Leu Ala Leu Gly Asp Asn Met

Glu Gly Tyr Thr Val Asp Leu Gln Val Ala Glu Tyr Leu Lys Ser Phe 455 460

Gly Arg Gln Val Leu Asn Cys Trp Ser Lys Gly Asp Ile Glu Leu Ser 475

Thr Pro Ile Pro Leu Phe Gly Phe 485

<210> 298

<211> 140

<212> PRT

<213> Chlamydia

<400> 298

Arg Ile Asp Ile Ser Ser Val Thr Phe Phe Ile Gly Ile Leu Leu Ala

Val Asn Ala Leu Thr Tyr Ser His Val Leu Arg Asp Leu Ser Val Ser 25

Met Asp Ala Leu Phe Ser Arg Asn Thr Leu Ala Val Leu Leu Gly Leu

Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly 55

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr 70

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys 105

His Ala Ser Trp Ile Ala Leu Ala Ser Tyr Phe Gly Gly Leu Ala Val

Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val 130 135 140

<210> 299

<211> 361

<212> PRT

<213> Chlamydia

His Gln Glu Ile Ala Asp Ser Pro Leu Val Lys Lys Ala Glu Gln Gln

- Ile Asn Gln Ala Gln Gln Asp Ile Gln Thr Ile Thr Pro Ser Gly Leu
- Asp Ile Pro Ile Val Gly Pro Ser Gly Ser Ala Ala Ser Ala Gly Ser
- Ala Ala Gly Ala Leu Lys Ser Ser Asn Asn Ser Gly Arg Ile Ser Leu
- Leu Leu Asp Asp Val Asp Asn Glu Met Ala Ala Ile Ala Met Gln Gly
- Phe Arg Ser Met Ile Glu Gln Phe Asn Val Asn Asn Pro Ala Thr Ala 90
- Lys Glu Leu Gln Ala Met Glu Ala Gln Leu Thr Ala Met Ser Asp Gln 105
- Leu Val Gly Ala Asp Gly Glu Leu Pro Ala Glu Ile Gln Ala Ile Lys 120
- Asp Ala Leu Ala Gln Ala Leu Lys Gln Pro Ser Ala Asp Gly Leu Ala 135 130
- Thr Ala Met Gly Gln Val Ala Phe Ala Ala Ala Lys Val Gly Gly 155 150
- Ser Ala Gly Thr Ala Gly Thr Val Gln Met Asn Val Lys Gln Leu Tyr 170
- Lys Thr Ala Phe Ser Ser Thr Ser Ser Ser Tyr Ala Ala Ala Leu 185
- Ser Asp Gly Tyr Ser Ala Tyr Lys Thr Leu Asn Ser Leu Tyr Ser Glu 195
- Ser Arg Ser Gly Val Gln Ser Ala Ile Ser Gln Thr Ala Asn Pro Ala
- Leu Ser Arg Ser Val Ser Arg Ser Gly Ile Glu Ser Gln Gly Arg Ser 230
- Ala Asp Ala Ser Gln Arg Ala Ala Glu Thr Ile Val Arg Asp Ser Gln
- Thr Leu Gly Asp Val Tyr Ser Arg Leu Gln Val Leu Asp Ser Leu Met 265
- Ser Thr Ile Val Ser Asn Pro Gln Ala Asn Gln Glu Glu Ile Met Gln
- Lys Leu Thr Ala Ser Ile Ser Lys Ala Pro Gln Phe Gly Tyr Pro Ala 295

Val Gln Asn Ser Val Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu

Arg Glu Phe Val Asp Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn 330 325

Ala Phe Arg Lys Gln Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile 345

Ala Ser Leu Phe Ser Gly Tyr Leu Ser

<210> 300

<211> 207

<212> PRT

<213> Chlamydia

<400> 300

Ser Ser Lys Ile Val Ser Leu Cys Glu Gly Ala Val Ala Asp Ala Arg

Met Cys Lys Ala Glu Leu Ile Lys Lys Glu Ala Asp Ala Tyr Leu Phe

Cys Glu Lys Ser Gly Ile Tyr Leu Thr Lys Lys Glu Gly Ile Leu Ile

Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu

Arq Asn Tyr Phe Arq Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp 105

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala 135

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu 150 155

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu · 185

Tyr Gly Pro Phe Leu Gln Ala Val Thr Trp Ser Gln Glu Lys Lys 200

<210> 301

<211> 183

<212> PRT

<213> Chlamydia

Ile Pro Pro Ala Pro Arg Gly His Pro Gln Ile Glu Val Thr Phe Asp

Ile Asp Ala Asn Gly Ile Leu His Val Ser Ala Lys Asp Ala Ala Ser 25

Gly Arg Glu Gln Lys Ile Arg Ile Glu Ala Ser Ser Gly Leu Lys Glu

Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu

Asp Lys Gin Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly

Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile

Prc Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg 100

Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser

Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala 135

Gln Ser Ala Ser Ala Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly 150

Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 170

Pro Pro Ala Gly Gly Ser Ala 180

<210> 302

<211> 232

<212> PRT

<213> Chlamydia

Met Thr Lys His Gly Lys Arg Ile Arg Gly Ile Gln Glu Thr Tyr Asp

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Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala Ile Asp Ile Leu Lys Gln - 25

Cys Pro Thr Val Arg Phe Asp Gln Thr Val Asp Val Ser Val Lys Leu

Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser 55

Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala

Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly 85 90

Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp

Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly 120

Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr

Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys 150 155

Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala 165 170

Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu 185

Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200

Leu Val Asn Phe Thr Ile Ser Ser Thr Met Gly Pro Gly Val Thr Val

Asp Thr Arg Glu Leu Ile Ala Leu 230 225

<210> 303

<211> 238

<212> PRT

<213> chlamydia

<400> 303

Ile Asn Ser Lys Leu Glu Thr Lys Asn Leu Ile Tyr Leu Lys Leu Lys 10

Ile Lys Lys Ser Phe Lys Met Gly Asn Ser Gly Phe Tyr Leu Tyr Asn 25 20

- Thr Gln Asn Cys Val Phe Ala Asp Asn Ile Lys Val Gly Gln Met Thr 35 40 45
- Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro
 50 55 60
- Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser 65 70 75 80
- Asn Asn Pro Ser Thr Asn Ala Ser Ile Thr Ile Gly Leu Asp Ala Glu 85 90 95
- Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly 100 105 110
- Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp 115 120 125
- Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn 130
- Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg 145 150 150 160
- Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val
- Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile 180 185 190
- Ala Ser Arg Met Glu Gly Gly Val Val Leu Ala Leu Val Arg Glu Gly
 195 200 205
- Asp Ser Lys Pro Tyr Ala Ile Ser Tyr Gly Tyr Ser Ser Gly Val Pro 210 215 220
- Asn Leu Cys Ser Leu Arg Thr Arg Ile Ile Asn Thr Gly Leu 225 230 235

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